SEEDS

SEEDS: The OARDC Research Enhancement Competitive Grants Program

Report of Progress for Calendar Year 2010

Ohio Agricultural Research and Development Center

BRINGING KNOWLEDGE TO LIFE

THE OHIO STATE UNIVERSITY OARDC

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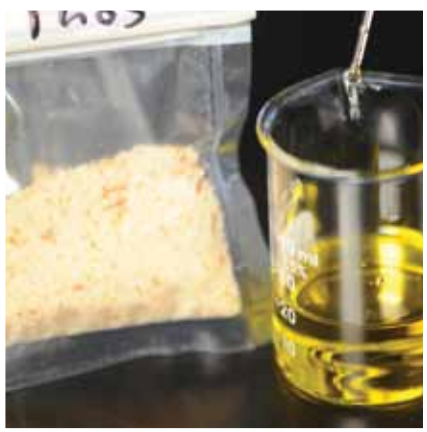

Current and Past Industry Partners

3-I
6062 Holdings LLC
AccuDX Inc.
Ag-Spectrum
Alltech
Alpaca Jack's Suri Farm
American Aggregates Corp.
American Berry Cooperative
American Coal Ashland Association
American Hosta Society
Ampac Seed Company
Antorchas Foundation
Archer-Daniels-Midland Company
Argus Control Systems Ltd.
Around the World Gourmet
Asgrow Seed Company
Athersys Inc.
Aviagen
BASF Plant Science GmbH Agrarzentrum Limburgerhof
Bass Endowment
Bayer Corporation
Bayer CropScience LP Environmental Sciences
Bedding Plants Foundation, Inc.
Berlin Natural Baker, Inc.
Biotechnology Research and Development Corporation
Boehringer Ingelheim-NOBL
British Columbia Greenhouse Growers' Association
British United Turkeys of America
California Avocado Commission
Camelid Health Foundation
Campbell R and D
Cargill Animal Nutrition Center
Cattlemen's Carcass Data Service
Center for Asceptic Proccessing and Packaging Studies
Central Ohio Hosta Society
Certified Angus Beef
Ciba Crop Protection
Cinergy
City of Columbus
Cognis Deutschland GmBH and Co.
Consortium for Plant Biotechnology Research
Cooper Farms, Inc.
Cultiva
Dairy Management, Inc.
Danone
DeVenture
Donlar Corporation
Dow Agrosciences
Dynal Biotech
E.I. DuPont de Nemours and Co.
Eagle-Picher Minerals, Inc.
Earthgro
Edstrom Industries, Inc.
Elanco Animal Health
Eli Lilly and Company
Farmland Industries
First Energy
Floriculture Industry Research and Scholarship Trust
Food Science Austrailia
Fremont Pickle Growers Association
Fruit Growers Marketing Association
Garick
General Chemical
George F. Ackerman Company
Great Lakes Hosta Society
Gregson Technologies, Inc.
Gustafson, Inc.
Harris Moran Seed Company
Hillshire Farm and Kahn's
Hirzel Canning Co.
Holmes Cheese Company
Holmes Cheese Table
Horticultural Research Institute
Iams Corporation
Infectech, Inc.
Ingredient Innovations International
Integrated Research Technology, LLV
J. Frank Schmidt Family Charitable Foundation
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Jatco, Inc.
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Kanter Associates
Kohlpyr
Kraft Foods Global, Inc.
Kurtz Brothers, Inc.
Lilly Research Laboratories
Lipha Tech, Inc.
Lipton Tomato Research Center
Loveland Industries, Inc.
Magical Farms, Inc.
Maple Leaf Farms, Inc.
Martel Biosciences Corporation
Merial Limited
MicroBio Limited
Mid-America Food Processors
Middlefield Cheese
Midtech
Midwest Regional Hosta Society
Ministry of Culture, Education, and Scientific Exchanges, Spain

MTD Products
National Fish and Wildlife Foundation
National Sea Grant Program
National Wildlife Federation
Natural Fiber Composites Corporation
North American Strawberry Growers Research Foundation, Inc.
Nourse Farms, Inc.
Novartis Crop Protection, Inc.
Nursery Growers of Lake County Ohio, Inc.
N-Viron International, Inc.
Ohio Bioprocessing Research Consortium
Ohio BioProducts Innovation Center
Ohio Corn Marketing Program
Ohio Dairy Farmers Federation, Inc.
Ohio Dairy Producers
Ohio Floriculture Foundation
Ohio Fruit Growers Society
Ohio Lawn Care Association
Ohio Nursery and Landscape Association, Inc.
Ohio Pork Producers Council
Ohio Poultry Association
Ohio Seed Improvement Research
Ohio Sheep and Wool Program
Ohio Soybean Council
Ohio Space Grant Consortium
Ohio Vegetable and Small Fruit Research and Development
Ontario Greenhouse Vegetable Growers
Optimum Quality Grains, LLC
Otterbein University
Outback
Park Foundation
Pennington Seed, Inc. Oregon Division
Petroseed
Pfizer
Pharmacia, Wyeth Ayterst Research
Philip Morris, Inc., Shared Solutions in Agriculture
Phycotransgenics
PIC USA
Pig Improvement Company
Pioneer Hibred International, Inc.
Polter Berry Farm
Protein Technologies International
Purity Foods, Inc.
Quality Liquid Feeds
Rainbow Treecare Scientific Advancements
Rainforest Phytoceuticals
Raven
Rhodia, Inc.
Roche Vitamins Inc.
Satloc
Schmack Bio Energy
Select Sires
Seminis Vegetable Seeds, Inc.
Small Farm Institute
Smathers-Oasis Company
Syngenta
The Chef's Garden, Inc.
The Garland Company, Inc.
The HANOR Company, Inc.
The Scotts Company and Subsidiaries
Theis Technology Inc.
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Introduction

SEEDS: The OARDC Research Enhancement Competitive Grants Program

The oldest industry known to humankind is still the one driving Ohio's economy. The economic powerhouse that is Ohio's agbioscience industry is unparalleled. Agriculture is the most distributed industry across Ohio with operations in every county. It accounts for $98 billion, or 11% of the state's total economic output and generates more than 984,000 Ohio jobs (15% of all employment in the state). No other economic engine comes close to making the kind of impact generated by agriculture, food, and the nursery and landscape industry. This massive industry employs one in every six Ohioans and supports a diversified and dynamic economic sector that touches the lives of everyone in the state. At any given time, there are over 600 research and development projects underway at The Ohio Agricultural Research and Development Center (OARDC).

The innovations produced in the agbiosciences are driving new, highly visible economic opportunities for American states, and the State of Ohio has been an early mover in recognizing the economic development potential of the industry. As the nation's largest and most comprehensive agbioscience research center, OARDC at The Ohio State University is a pioneer of cutting-edge innovation, establishing itself among the essential drivers of Ohio's economy. OARDC is the research and development hub for agbioscience research in Ohio and serves as the state's signature research center for realizing progress in all significant aspects of the biobased economy. OARDC's Ohio spending impacts generated 1,609 jobs; $156.3 million in economic output; $59.2 million in personal income for Ohio residents, and $5.5 million in state and local taxes. In a knowledge-driven economy, intellectual property is perhaps the most valuable property that can be produced.

Due to the changing nature of economic and societal trends, agriculture, food, and the green industry depend on innovators and researchers to generate new processes and products. Ohio's largest industry increasingly links with other industries to take on common challenges and opportunities in key areas such as environmental restoration, organic agriculture, and the development of biorenewable sources such as energy, fuel, and industrial goods. Opportunities abound in biobased industrial products, and agbiosciences holds the promise of stimulating new economic growth across existing and new economic sectors throughout the state.

The ultimate goal of SEEDS: The Research Enhancement Competitive Grants Program is to address the differing challenges and vast opportunities of Ohio's largest industry. SEEDS encourages excellence in OARDC research by promoting exploration that is consistent with the mission and vision of The Ohio Agricultural Research and Development Center and by encouraging connections across disciplines, with industry, and with other external partners.

Established in 1996 and supported by an appropriation from the Ohio General Assembly to OARDC, SEEDS: The Research Enhancement Competitive Grants Program is unique among US state-assisted universities. In fostering high-quality research among OARDC and The Ohio State University College of Food, Agricultural, and Environmental Sciences (CFAES)-supported scientists, SEEDS enables those scientists to collect the preliminary data needed to give them a competitive edge in national programs, and it provides them with leverage to attract industry support.

OARDC's SEEDS program is just one of the many ways in which The Ohio State University's innovative research and development connects to industry and community on an eminent global scale. Currently, Ohio State is ranked 19th among the nation's public universities and has been among the top 25 public research universities in each *U.S. News & World Report* ranking. According to the National Science Foundation's assessment of sponsored research expenditures, Ohio State ranks among the top 10 public research universities in the country.

OBJECTIVES

SEEDS was created to encourage partnerships with industry and other stakeholders and to increase the competitiveness of OARDC/CFAES scientists in extramural grant programs. While these objectives remain as the program's cornerstone, SEEDS has grown to include a total of seven objectives:

- Increase the competitiveness of scientists in extramural grant programs.
- Encourage partnerships with industry and other stakeholders.
- Encourage the development of interdisciplinary teams.
- Encourage international collaborations.
- Support the exploration of enterprises that are potentially new to Ohio.
- Provide undergraduate students with research experience.
- Provide graduate students with the opportunity to take part in the grant-writing/review process.

By providing SEEDS money to develop the necessary preliminary data for a strong grant application or by matching funds to leverage external funding, SEEDS has proved to be a valuable program for scientists in the College of Food, Agricultural, and Environmental Sciences. Within the context of our global society, the SEEDS program looks forward to continued success and new partnerships with industry and other collaborators in Ohio and throughout the world.

PROGRAM ACHIEVEMENTS

Overall, SEEDS or SEEDS-funded researchers and/or graduate students have . . .

- supported research projects at around $11,753,760 in all categories and has received close to $67,000,000 in matching and extramural funding—a return of about $5.25 for each dollar invested.
- invested $2,555,752 in projects requiring matching funds, generating $4,722,165 in industry matches—a return of $6 for every dollar invested over the last five years.
- enabled scientists to establish collaborations with colleagues from Africa, Argentina, Australia, Belgium, Brazil, Chile, France, Ireland, Italy, Mexico, New Zealand, Norway, the Philippines, Switzerland, Taiwan, Uganda, and Zimbabwe.
- applied for nine US patents, using results of initial findings. Three patent applications have been granted, and three licensing agreements have been obtained.
- published a total of 727 peer-reviewed scientific manuscripts, abstracts, popular press articles, bulletins, and/or book chapters and made more than 1,307 presentations throughout the world.
- produced 44 doctoral dissertations and 77 masters' theses.

Achievements by Objectives

Objective 1: Increase the competitiveness of scientists in extramural grant programs.

The Seed Grant Competition and the Agency External Competitions specifically address Objective 1. However, all the other competitions may result in additional funding from outside sources.

Of the 31 projects completed and reported in calendar year 2010, $2,071,472 was generated in extramural funding. Over the life of SEEDS, 346 projects have been completed and $52,885,846 has been generated extramurally.

Over the life of SEEDS, OARDC has invested $684,067 in matching funds for Agency External Grants, which has generated $8,637,193 in extramural funding—a return of more than $12 for each dollar invested in the SEEDS category.

Objective 2: Encourage partnerships with industry and other stakeholders.

The Matching and Industry Small Grant Competitions address Objective 2.

Of the eleven grants requiring at least a dollar-for-dollar match and completed during calendar year 2010, OARDC provided a total of $248,654 while industry matched those dollars with $461,181.

Over the life of the program, OARDC has provided $2,555,752 toward Matching and Industry Small Grants while industry has matched these dollars with $4,722,357—a return of close to $2 on each dollar invested.

Objective 3: Encourage the development of interdisciplinary teams.

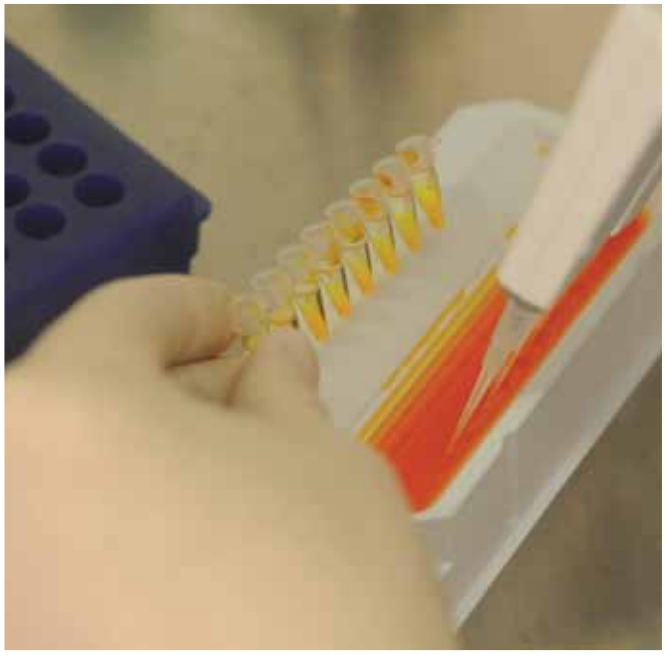
The Interdisciplinary Team Competition specifically addresses Objective 3.

During calendar year 2010, five interdisciplinary teams completed projects. These teams reported receiving $600,000 in extramural funding.

Over the life of the program, six colleges and 23 departments have participated in this category of competition, with OARDC investing $5,636,911 and teams competing successfully and reporting $15,477,006 in extramural funding—a return of $2.75 on each dollar invested.

Objective 4: Encourage international collaborations.

All competitions may have an international collaboration component, and international relationships are encouraged. OARDC scientists have collaborated with scientists from Africa, Argentina, Australia, Belgium, Brazil, Chile, France, Ireland, Italy, Mexico, New Zealand, Norway, the Philippines, Switzerland, Taiwan, Uganda, and Zimbabwe.



Objective 5: Support the exploration of enterprises that are potentially new to Ohio.

New Enterprises are considered to be crops, animals, products, goods, and services that currently are not produced for biological, physical, cultural, processing, economic, or social reasons. The New Enterprise Competition is designed to explore new enterprises and to eliminate the barriers that constrain existing ones.

The New Enterprise Competition has received 33 applications; twelve have been funded.

Funded projects include the following:

- New Commodity Enterprises in Ohio—Evaluation and Education
- Development of New Biological Products for Slug Control
- Direct Conversion of Agricultural Wastes to Electricity Using Rumen Microbes in Microbial Fuel Cells
- Domestication and Commercialization of Taraxacum—A New Crop to Fuel Ohio's Agricultural and Rubber Industry
- Cultivar Evaluation and Development of Hard Winter Wheat for Organic Production in Ohio to Meet New Marketing Opportunities for Ohio's Organic Farmers, Millers, and Bakers

Objective 6: Provide undergraduate students with research experience.

A total of 68 applications to the Director's Undergraduate Research Program have been received. Forty-three applicants have received awards.

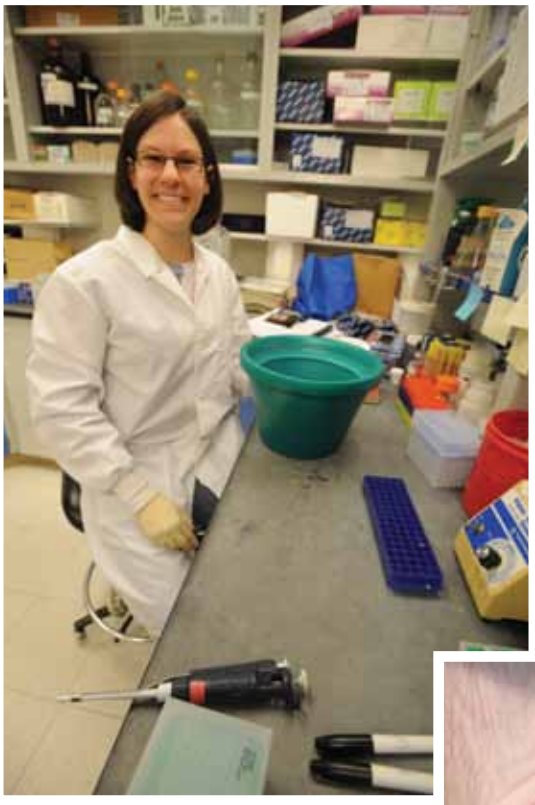
The Director's Undergraduate Research Program provides undergraduate students with a professional grant-writing, research, and reporting experience. Projects are designed, submitted for review, and carried out with a faculty mentor. Once a project is completed, students take an independent studies class to write their research report in the form of a scientific journal article, using their faculty advisor as an editor. Some of these reports get published. In addition, many students present their research at professional meetings and at competitions such as the Denman Undergraduate Research Forum, a university-wide program presented by The Ohio State University Office of Research and The University Honors and Scholars Center.

Objective 7: Provide graduate students with the opportunity to take part in the grant-writing/review process.

A total of 432 master's and doctoral students have submitted proposals in this competition. One hundred seventy-six projects have been awarded. The graduate competition is run exactly like a federal competition. Graduate students who receive awards are asked to serve on a panel to review applications in the following year's competition. This experience provides students with the opportunity to develop their skills in grant-writing and reviewing—skills that are essential to their careers.

Interdisciplinary Team Competition

The Interdisciplinary Team Competition, funded at a maximum $100,000 level, is designed to stimulate new collaborative partnerships in multiple departments and colleges or build on existing programs of excellence. Interdisciplinary research provides expertise over several disciplines, bringing a more holistic approach to research questions and problems.



Identifying Berry Antioxidants That Help Fight Cancer

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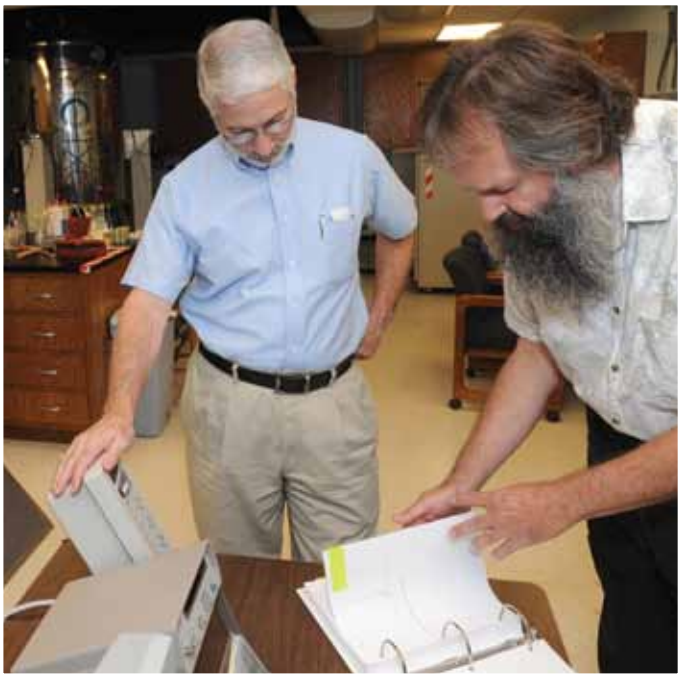
Ray Miller, Horticulture and Crop Science
Joseph C. Scheerens, Horticulture and Crop Science
Joshua A. Bomser, Human Nutrition
Faith U. Wyzgoski, Chemistry

The antioxidants that are present in many fruits and vegetables help protect produce from damage due to unfavorable environmental conditions or from attack by bacteria, fungi, insects, and other pests. Through decades of research, health professionals have determined that, when included in a person's diet, antioxidants might also help protect against common diseases—such as cardiovascular disease and various cancers—associated with age. Based upon this ever-increasing evidence, governmental and public agencies have developed programs such as the USDA's Food Guide Pyramid and the 5 A Day For Better Health Program to encourage Americans to eat more antioxidant-rich foods. Strawberries, raspberries, blackberries, and blueberries are among the 50 highest antioxidant-containing foods in the American diet both by weight and on a per-serving basis. Berry antioxidants belong primarily to a class of health-beneficial compounds known as polyphenols, which have been recently advertized on TV as the important antioxidants in Concord grape juice products. There are several hundred different polyphenols in berries, and polyphenols can affect berries' appearance, flavor, and other quality attributes such as the health benefits received from eating berries.

The goal of this research was to develop a method by which researchers could identify specific polyphenolic compounds in a very complex mixture, as well as identify the compounds' optimum relative proportions that are responsible for the health benefits of berries. The relationship between diet and disease is complex, and the task was difficult to achieve. A team of scientists with very different backgrounds (including horticulturists, plant physiologists, chemists and biochemists, food scientists, human nutritionists, medical researchers, and statisticians) was assembled. The underlying principle of the method is that variability in composition among berry samples will result in a variable biological response in living tissues with respect to the disease process. Using sophisticated tools of chemistry (nuclear magnetic resonance and chromatography), biology, medicine, and statistics, the team screened large numbers of berry samples and compared chemical differences with disease responses.

Joseph C. Scheerens



Mathematical models were developed to match the ability of black raspberry extracts to prevent the growth of human colon cancer cells with amounts of individual polyphenolic compounds in each extract. Black raspberries have been extensively examined by medical scientists in The Ohio State University College of Medicine and the College of Dentistry for their beneficial effects against cancers of the mouth, throat, and digestive system. Fundamental research has progressed to initial clinical trials with cancer patients. Human colon cancer cell assays were chosen for the development of the model because they can be cultured with relative ease.

The study has produced valuable information that is important on several levels. From a biological standpoint, it has advanced scientists' understanding of the impact of plant extracts on cancer cell growth. It offers the promise of additional insights into the disease process. From a medical perspective, this information can be used to ensure the potency and reproducibility of products developed to clinically treat specific cancers of the mouth, esophagus, or colon. Such information will also be important for black raspberry producers. By choosing specific varieties and managing growing and handling methods, producers may have the opportunity to optimize levels of specific beneficial compounds, and thus, provide a more healthful product for the consumer. Finally, the method is adaptable. The same technique could be used to determine the important compounds driving any system where variability in sample chemistry results in a variable biological response.

Urbanization and Lake Erie Tributary Water Quality: A Dynamic Spatial Model with Policy Scenarios

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David Culver, Evolution, Ecology, and Organismal Biology
Ciriyam Jayaprakash, Physics
Jay F. Martin, Food, Agricultural, and Biological Engineering

The Lake Erie watershed is the most urbanized and populated watershed of the Great Lakes. Of the 11,649 square miles in the Ohio Lake Erie watershed, over 78% of the land has been altered from its original use and more than 90% of the original coastal marshlands have been filled or converted to other uses. Urbanization has increased the sediments and toxic chemicals entering the tributaries in many of Lake Erie's watersheds and has increased the flow rate of runoff due to increases in impervious surfaces and channelization of streams and rivers. Despite these impacts on water quality, little is known about how to design and implement policies that will induce a pattern of land use that both protects water quality and balances the needs of individuals and society. A challenge here is that land use patterns are the cumulative result of individual land use and location decisions by which government entities typically have limited control. Because of these limited controls, policies must often seek to provide the right set of incentives to encourage landowners, households, and businesses to make land use and location choices that correspond to the well-being of society and the natural ecosystem. Doing so requires an understanding of the factors that influence household demand for residential location as well as an understanding of how the Lake Erie ecosystem is impacted by urbanization and what the trade-offs are between unregulated urban development and a healthy Lake Erie ecosystem.

Ohio Agricultural Research and Development Center scientists developed two conceptual models of human-lake interactions. First, a regional model was developed, allowing them to explore how lake amenities influence regional growth and, in turn, how this growth impacts the lake ecosystem. Second, a more detailed ecological model of Lake Erie was developed; it considered how public support for Lake Erie protection policies could influence management decisions and water quality. The results from the first phase of the research show that a representative household positively values Lake Erie water quality and that improvements in nearby water quality are positively capitalized into housing values. Using the second-stage hedonic model results, it was found that a mean household in the study region is willing to pay $1,104 for a 1% improvement in average water clarity and $1,810 for a 1% improvement in water quality, as measured by a reduction in the level of fecal coliform. Further improvements in water clarity and quality result in higher benefits to homeowners. For example, a reduction in fecal coliform. The results from the simulation of the regional human-lake model show that economic-ecological interactions fundamentally alter the regional economic dynamics and influence the degree to which the system can maintain a healthy economy and ecosystem.

Elena Irwin

This research has led to additional research—currently being sponsored by the National Oceanic and Atmospheric Administration through the Ohio Sea Grant Program—on housing location decisions and regional growth.

An On-Farm Tool for Management of Nitrogen Nutrient Loss and NH3 Emission from Animal Manure

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Harold Keener, Food, Agricultural, and Biological Engineering
Jon Rausch, Food, Agricultural, and Biological Engineering
William P. Weiss, Animal Sciences
J. David Latshaw, Animal Sciences

Lingying Zhao

Ohio is the second largest egg-producing state and the eleventh largest dairy milk-producing state in the nation, with annual revenues or income of about $607 million for egg production and $552 million for dairy products. Ammonia (NH3) emission from concentrated livestock feeding facilities, which accounts for 80% of NH3 emission to the atmosphere, has become a significant environmental issue. Recently, the U.S. EPA explicitly announced that all animal feeding operations (AFOs) need to be in compliance with federal air quality laws and regulations. Under the Environmental Planning and Community Right-to-Know Act (EPCRA), AFOs are required to report any emission of NH3 exceeding 100 pounds in a 24-hour period. The increased public concerns and the federal air quality regulations make poultry and dairy industries very vulnerable to lawsuits because the dairy industry emits about 46% and the poultry industry emits about 24% of the nation's total NH3 emissions. However, farmers are unaware of the quantity of NH3 emitted from their operations. This issue is a major constraint to the compliance, profitability, and growth of livestock industries. This study was proposed to supply the Ohio poultry and dairy industries with a powerful tool with which to efficiently manage NH3 from their facilities, make wise decisions on adoption of mitigation technologies, stay in compliance with federal air quality regulations, and maintain viable and sustainable production operations.

To estimate air emissions from AFOs, field air emission measurement is generally accepted as the most accurate method for quantification of NH3 emissions. However, it is very expensive, time-consuming, and limited to specific facilities (e.g., fan-ventilated buildings). The nitrogen mass balance method is fundamental and has been used widely to predict NH3 emission and validate for other NH3 emission estimation methods, but it needs precise tracking of mass flows including waste flow on farms, which is not a typical practice and is very difficult. This study presents an innovative mass balance modeling approach, which tracks more than two nutrient balances to eliminate the need for precise measurement of waste flow rate. This method only requires tracking of regular operation parameters as inputs to predict and manage NH3 emissions from animal farms.

Through this research, NH3 emission management tools to evaluate NH3-N emissions from dairy and poultry layer productions have been developed based on conceptual Nitrogen/Ash or Nitrogen/Phosphorus mass balances theory. Baseline nutrient mass balances have been verified through studies of small-scale experimental layer and dairy farms. Models reflecting on-farm nutrient balances and the associated spatial and temporal variations in nutrient contents of commercial dairy and layer operations have been established. The models have been converted to a producer-friendly, web-based decision-making/support tool for producers, governmental agencies, and livestock and poultry professionals to effectively manage NH3 emissions.

An alternative mass balance model for manure-belt layer facilities has been developed and the model can be used to effectively estimate NH3 emissions from manure-belt poultry layer houses. Web-based decision-making tools for poultry manure-belt layer facilities and sand-bedding dairy houses have been developed. The tools will lead to efficient management of NH3 emissions, adoption of management practices and mitigation technologies, reduction of environmental and health impacts of the industries, increased manure value, and viable and sustainable production operations.

An Extension program on understanding the knowledge of and tools for nitrogen flow, ammonia emission, and control of nitrogen loss will be developed to disseminate the research findings to stakeholders. A proposal will be submitted to the 2011 Agriculture and Food Research Initiative grant program to apply the mass balance method for analysis of nutrient flows, ammonia emission, and methane emissions of a diary farm for assessment of its environmental impact and carbon footprint.

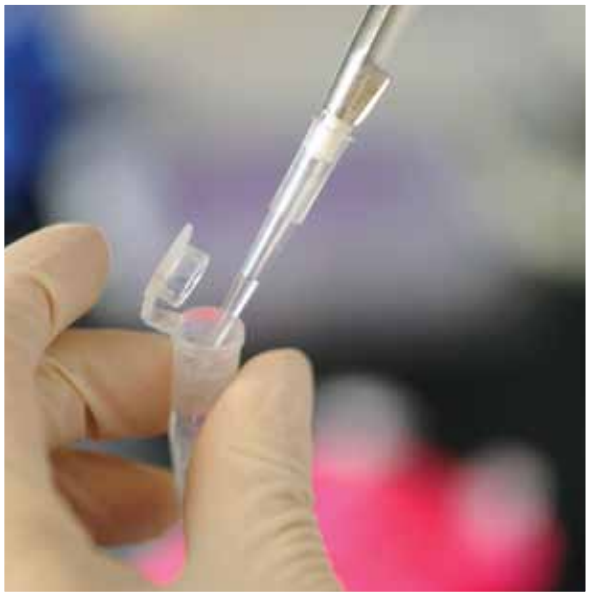
Virulence Determinants of Entomopathogenic *Photorhabdus* and *Xenorhabdus* Bacteria

Parwinder S. Grewal, Entomology
Gireesh Rajashekara, Food Animal Health Research Program
Donald Dean, Biochemistry

Entomopathogenic bacteria *Photorhabdus* and *Xenorhabdus* form symbioses with the entomopathogenic nematodes (commonly known as roundworms) *Heterorhabditis* and *Steinernema*, respectively. These bacteria not only have similar biology but are also phylogenetically related based on 16s rDNA sequence identities. They naturally colonize intestines of the nematode infective juveniles, which invade susceptible insects to release the bacteria. The bacteria multiply, killing the insect host within 24–48 hours and converting the cadaver into a food source suitable for nematode growth and reproduction. After 1–3 rounds of nematode reproduction, the bacteria recolonize the emerging infective juveniles, ensuring their transmission to a new host.

Parwinder S. Grewal

As many of the insect species susceptible to the bacteria-nematode partnership are crop pests, understanding the genetic basis of the tripartite association between nematode-bacterium-insect is expected to enhance biological pest control applications of entomopathogenic nematodes and bacteria in agriculture. Using a selective capture of transcribed sequences, comparative hybridization, quantitative real-time PCR and mutagenesis, scientists determined global gene expression of *Photorhabdus temperata* and *Xenorhabdus koppenhoeferi* upon infection of the white grub *Rhizotrogus majalis*.



Researchers found 40 genes in *P. temperata* and 39 in *X. koppenhoeferi* to be potentially involved in grub infection. In the future, the functions of these genes will be studied to determine the relative contribution of each gene to the virulence of the bacteria to the grub.

Study on the Pro-Myogenic and Anti-Adipogenic Functions of Delta-Like Protein in Chicken

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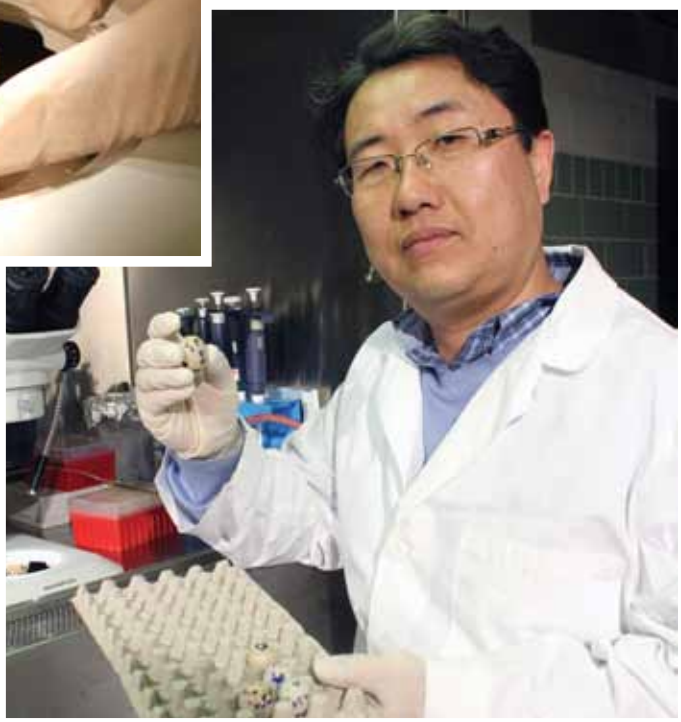
Sandra G. Velleman, Animal Sciences
Martha A. Belury, Human Nutrition

Poultry production is of increasing economic importance in the U.S. due to the upward trend in worldwide poultry consumption. Ohio's poultry and egg production, valued at more than $866 million, ranks fifth among agriculture industry segments in Ohio. Ohio has more than 57.5 million broilers valued at more than $147.5 million and ranks tenth in turkey production, with 6 million turkeys valued at $133.6 million. The net effect of improvement in lean muscle mass and reduced carcass fat deposition tends to be correlated with improved feed efficiency. Under current conditions, the economic value of a gain in breast yield and feed conversion to the poultry industry are huge. Thus, the proposed goal of identifying a factor and a dietary manipulation that will enhance muscle growth and decrease fat accretion is of significant financial importance to poultry producers in Ohio.

Improving muscle growth and decreasing fat accretive poultry species are essential in maximizing poultry production. Therefore, identification and characterization of factors that enhance muscle growth and reduce fat mass will be necessary to achieve this goal. Recent studies have shown that Delta-like protein 1 (*DLK1*) has muscle-enhancing affects and the ability to reduce fat accretion in rodents. However, the role of *DLK1* in the development of muscle and fat tissues has not been studied in chickens.

The objective of this project was to determine whether the 1) amount of *DLK1* is associated with different rates of muscle growth in different genetic lines of chickens (muscle dystrophy, normal control) during muscle regeneration; 2) greater amount of *DLK1* production can inhibit fat accumulation in fat cells; and 3) feeding of conjugated linoleic acid (CLA) results in a reduction in fat mass of chickens.

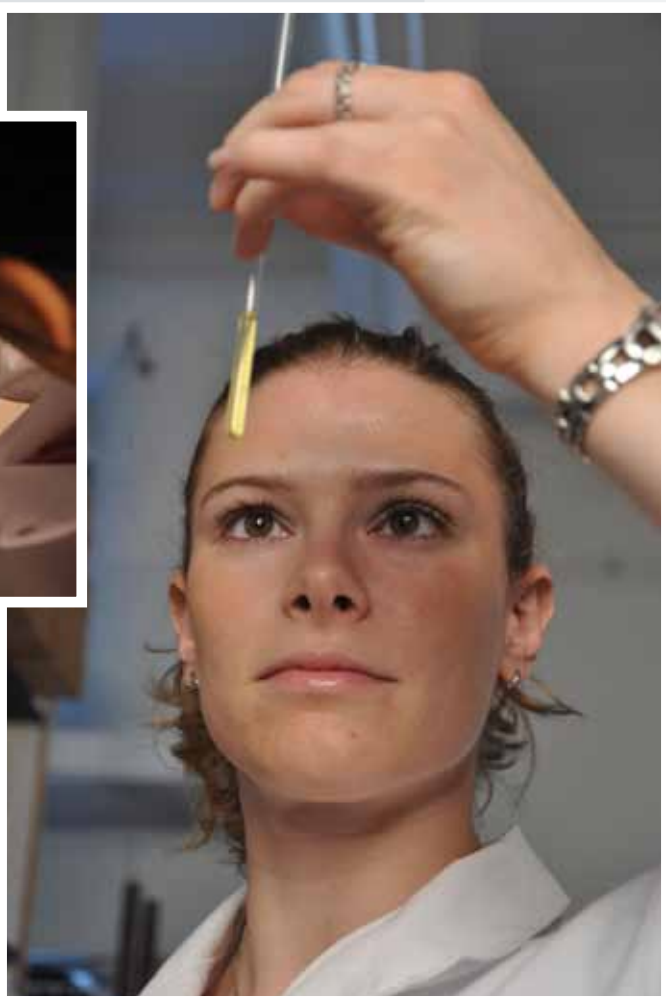
The study found that chickens with muscle dystrophy showed delayed muscle regeneration with a lagged appearance of myogenic markers. However, a higher amount of *DLK1* was found during muscle regeneration in muscle dystrophy chickens compared to control chickens. The data suggest that *DLK1* may have an important role in the early stage of muscle development. To date, scientists have made a virus-mediated gene transfer in chicken cells for further elucidating the role of *DLK1* in muscle growth and development. Development of the viral vector for gene transfer in poultry was successful, and the results were published. Supplementation of CLA is known to cause weight loss by reducing fat in humans and rodents. Although its effect is controversial in chickens, researchers found that CLA supplementation could reduce fat content in the broiler chickens. Researchers are currently investigating whether the amount of *DLK1* in fat tissue is associated with the loss of fat by CLA feeding. The proposed research could provide a basic understanding of the *DLK1*-controlling adipose and muscle development, and potentially serve as the intervention for maximization of muscle growth and minimization of fat accretion in poultry. The data from this study was successfully used to receive both an international competitive research grant sponsored by the Global Research Network Program and an Agriculture and Food Research Initiative grant. The data was also published in several journals, including *Poultry Science*, *Journal of Animal Science*, and *Lipids*.



Kichoon Lee

Matching and Industry Small Grant Competitions

The Matching and Industry Small Grant Competitions are specifically designed to develop partnerships with private industry and non-profit foundations. Industry Small Grants provide up to $6,000 from the SEEDS program while Matching Grants provide up to $50,000. Investigators are required to obtain at least a dollar-for-dollar match from industry for both of these competitions.



Development of an Integrated Management System for High-Quality Organic Spelt to Meet New Marketing Opportunities in Ohio

Deborah H. Stinner, Entomology
Brian B. McSpadden Gardener, Plant Pathology

Deborah H. Stinner

This study addressed a need to develop an integrated organic management system that would optimize variety, fertility, and disease management for production of organic spelt in order to meet the high-quality baking standards of an important new market in Ohio. Spelt is an important crop for many organic farmers both nationally and in Ohio. About one-third of all U.S. spelt is grown on certified organic land. Ohio is one of the leading producers. An ancient grain, with archaeological evidence dating to 4700 BC, spelt is hulled wheat, classified as a subspecies of bread wheat. There are many reports of wheat-sensitive persons who tolerate spelt, and this has created a marketplace niche for spelt products. Ohio is home to one of the nation's largest spelt companies, Berlin Natural Bakery (BNB). Located in Berlin, Ohio, the company pioneered the spelt movement in the U.S. in 1990; BNB goes to great lengths to ensure that its spelt products are free of wheat contamination. Most of the company's one million pounds of spelt grain needed to cover its annual production is sourced from Germany, which offers an extremely high-quality, chemical-free spelt of a proprietary variety (Franckenkorn) that is deemed superior in genetic purity and baking quality to the organic spelt typically produced in Ohio. The breeder of Franckenkorn has developed a management system for its contracted production in the Black Forest region of Germany. U.S. farmers are not allowed to grow the spelt and as a result, Ohio's spelt producers have lost a significant market.

Researchers began working with BNB on this challenge in 2003, but they were not allowed to work with the German variety at that time. When heating prices skyrocketed worldwide, the price of importing the German spelt doubled. This situation created an urgent need for more affordable domestic spelt of appropriate genetic purity to supply the company's existing and growing needs. The owner of BNB identified an old Swiss variety (Oberkulmer) as a variety of interest. As a result, all of the research for this study was done with Oberkulmer.

There are two main factors—besides variety—which can influence the baking quality and safety of bread grains: spelt high-protein levels and good disease control. The objective of this project was to investigate the effects of these two management factors along with fertility and seed treatment for disease management on the production and quality of the organic spelt that was of key interest to BNB. In conventional production, it is well known that added fertility—particularly, nitrogen topdressed in the spring—is critical for good grain protein levels. Organic farmers do not typically add fertility to spelt. In addition, an important part of the quality of Franckenkorn spelt has to do with disease management. Organic farmers in Ohio are just beginning to learn the importance of good disease management to ensure the food safety of organic bread grains such as spelt, but shelf-ready organic products comparable to conventional fungicides are not available. Because this research was done on certified organic research land, only certified, organic-approved products could be used. A commercially available certified organic poultry manure product was used along with a biological control seed treatment developed by the co-investigator, containing biological control strains of bacteria.

In the fall, an experiment was established with three fertility treatments with and without the biological control seed treatment with the Oberkulmer variety. The results suggested that fertility addition of 0.5 tons per acre of the poultry manure product applied in the fall at planting and in the spring as a topdress would be sufficient to improve yields by 10–15% and protein by 0.4%. Addition of more fertility in the fall did not support additional growth or better protein levels. No significant effects of the seed treatment were measured, but results indicated that seed treatment might be important when fertility is not optimal and it might speed early crop development.

Future plans include conducting experiments to look at the effects of different sources of organic fertility (particularly disease-suppressive composts) on grain production, disease and insect resistance, and specific proteins and starches that are functionally important in bread baking and processing.

Celiac Disease and Gluten Intolerance: Research and Development for a Gluten Replacement in Bakery Products, Phase I

Yael Vodovotz, Food Science and Technology

For individuals afflicted with celiac disease, gluten is toxic. However, gluten is also the structural protein in wheat that gives bread its open-cell crumb structure and flexibility. For celiac patients, the consumption of wheat products causes an allergic reaction that reduces nutrient absorption and increases the risk of a nutrient-related disease such as anemia. Affecting an estimated 1 in 133 individuals in the U.S., the only treatment for celiac disease is a life-long adherence to a gluten-free lifestyle. Nevertheless, celiac sufferers crave many gluten-containing foods such as bread and therefore, a need exists to provide high-quality, gluten-free baked goods. Food gums are commonly used to bind water and to provide additional structure to gluten-free bread. However, published research has not studied the gums alone; rather it focuses only on the gums in conjunction with alternative proteins contributing to the overall variability of the gum behavior.

The objective of this research was to observe the physicochemical behavior of adding gum to a model rice cassava bread. The study continued with the investigation of the interaction between starch, proteins (soy protein isolate and egg whites), and water in the optimal gum-added rice cassava bread formulation. This was necessary since there is a great variability in previously published research on the performance of gums in gluten-free bread. The information obtained was to be used to create a superior gluten-free baked product that can be commercialized.

In order to analyze the behavior of gum in rice cassava dough, two hydroxypropyl methylcelluloses (HPMC) and xanthan gums were investigated at 0, 2, 3, and 5%. The properties of the dough were first analyzed by comparing the flow behavior and water binding profile to traditional bread dough. The dough was then baked and the loaf quality was measured using the same quality standards as traditional bread.

It was finally determined that while HPMC improved the loaf quality of the rice cassava bread, it was still of lower quality than traditional bread. While the addition of proteins and HPMC improved the rice cassava bread, there was an antagonistic interaction between the soy protein isolate and egg white solids reducing the water binding ability and functionality of the HPMC. It was not until egg white solids became the primary structural protein that the loaf quality improved comparable to traditional bread.

The characterization of model rice cassava dough with the addition of gums and alternative proteins has provided valuable insight into gluten-free applications and a point of reference for future work. Nonetheless, enough was learned to contribute to an improved product for Around the World Gourmet, a company that specializes in all-natural, gluten-free, low-sugar, low-sodium gourmet products. As a result of this research, Around the World Gourmet can accelerate the commercialization of its gluten-free bread.

Yael Vodovotz

Quantification of Isoflavone Transfer from Feed into Hen Tissues

M. Monica Giusti, Food Science and Technology
J. David Latshaw, Animal Sciences

Isoflavones are compounds found in certain plants—mainly in soy—that have been widely studied because of their phytoestrogenic activity. They have been associated with many health benefits such as cancer prevention, reduction in menopausal symptoms, and bone mass increases among others. However, in some instances, their impact on health seems detrimental. Consumers are becoming more selective in regard to their dietary intake: some seek soy enhancements while others prefer foods free of estrogenic compounds.

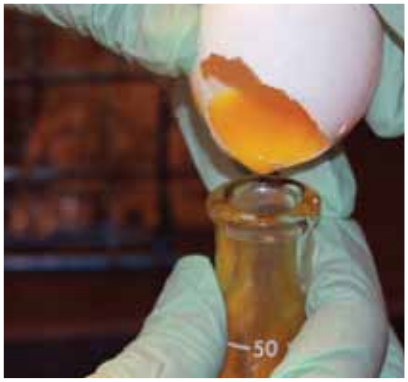
In a first study, researchers determined that soy isoflavones as well as the more biologically active phytoestrogen equol can be found in commercial egg products in different concentrations. Therefore, eggs constitute an additional source of phytoestrogens in the human diet. In this study, the objective was to evaluate the transfer and accumulation of isoflavones and a metabolite, equol, into hen eggs and into tissues of birds that were fed a diet of different soy and isoflavone content.

Hens were provided three different dietary interventions: a soy-free diet, a regular soy-based diet (25% soybean meal), and a diet enhanced with isoflavones (25% soybean meal plus 500mg isoflavones/100g feed). Eight laying hens were used as a baseline. Isoflavones found in this baseline period were attributed to soybean meal present in the regular feed. Forty-eight laying hens were subjected to the dietary interventions for 30 days. Eight cages (two hens per cage) were used per treatment. Eggs were collected over the 30-day period and evaluated for soy isoflavones and the isoflavone metabolite, equol. On day 30, tissues were collected and analyzed. Eggs from the baseline tests contained trace amount of isoflavones.

As expected, isoflavones were found at first in all eggs analyzed at concentrations comparable to those found in commercial eggs. However, isoflavones disappeared in just 10 days of soy-free feed consumption. Eggs from hens in the control diet (25% soybean meal) showed an average of 20.27ug isoflavones/100g egg yolk. Finally, a significant increase in isoflavone content was observed in eggs from hens subjected to the isoflavones-enhanced feed: values up to 175ug isoflavones/100g were reached within two weeks of treatment. This concurs with egg yolk development time. Tissue samples including muscle, liver, heart, kidney, and ovaries were also analyzed. Livers, kidneys, hearts, and muscles all contained varying concentrations of isoflavones.

Researchers concluded that poultry diet can be altered to produce isoflavone-free eggs or designer eggs to provide an additional source of isoflavones. This information will be very useful for the poultry industry as well as for consumers who are concerned about estrogenic compounds in their diet.

M. Monica Giusti



Development of Combined Liquid and Solid-State Anaerobic Digestion Process for Methane Production from Sewage Sludge and Organic Waste

Yebo Li, Food, Agricultural, and Biological Engineering

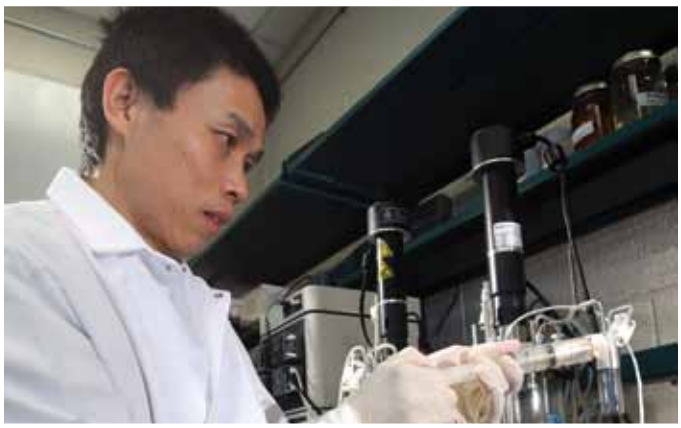
The process of anaerobic digestion (AD) involves methanogenic decomposition of organic matter under oxygen-free conditions. It involves a consortium of anaerobic microorganisms that transform organic matter into biogas. Typically, the methane content of biogas is about 40–70%. These liquid phase digesters are usually operated with a total solid concentration of 0.5% to 15%. Anaerobic digestion effluent, which typically contains high amounts of ammonium, phosphate, suspended solids, and dissolved solids, has generally been applied as a fertilizer. However, there often is not enough agricultural land available to use the digested effluents within an economical transportation limit.

In contrast to liquid AD systems, solid-state anaerobic digestion (SS-AD) systems generally operate with 15–40% total solids, which are suitable for processing solid organic waste. Some of the advantages of SS-AD systems include requirements of a reactor with a smaller capacity, less energy for heating, no processing of energy for stirring, and reduced material transportation costs. Due to the lower water content, an SS-AD system's digestate can be used as fertilizer or it can be composted, which is much easier to handle than the effluent of a liquid AD system. The disadvantages of conventional SS-AD systems include the requirements of large amounts of inoculum, a much longer retention time (three times longer than liquid AD systems), and nitrogen source supplements when lignocellulosic biomass is used.

Using the Integrated Anaerobic Digestion System (iADs) technology developed in this project, liquid AD effluent was mixed with lignocellulosic biomass such as corn stover, wheat straw, leaves, and yard waste and fed into the SS-AD reactor. The liquid AD effluent functioned effectively as inoculum and nitrogen source, and the results showed that the retention time can be reduced to 20–30 days. The biogas produced from the SS-AD process is collected and directed to the combined heat and power (CHP) unit to be shared with the liquid AD system.

The goal of this project was to develop a scalable, combined liquid AD and SS-AD process for the treatment of effluent of

Yebo Li



liquid AD and biogas production from lignocellulosic biomass. The initial objectives of this project were to (1) develop a thermophilic SS-AD process for treatment of organic solid waste and liquid AD effluent and (2) evaluate the pathogen reduction and fertilizer value of SS-AD digestate.

Investigators studied the performance of SS-AD of corn stover, wheat straw, and leaves, using effluent of liquid phase AD as inoculum and a nitrogen source. Approximately 32% more polysaccharides were converted at thermophilic conditions (55°C) than that obtained at mesophilic conditions (37°C), resulting in 37% more biogas production. Among the feedstocks studied, the highest biogas yield of 403.7L/kgVS feed was obtained at a C/N ratio of 21.4 and a temperature of 55°C with corn stover.

Researchers investigated the effect of alkali pretreatment on the biogas production from corn stover through SS-AD. Different NaOH loadings (1, 2.5, 5.0 and 7.5% w/w) were tested for solid-state pretreatment of corn stover. Lignin degradation during pretreatment increased from 9.1 to 46.2% when NaOH concentration increased from 1.0 to 7.5%. The NaOH-treated corn stover was digested using effluent of liquid AD as inoculum and a nitrogen source. NaOH loading of 1% did not cause significant improvement on biogas yield. The highest biogas yield of 372.4L/kgVS was obtained with 5% NaOH-treated corn stover, which was 37% higher than that of the untreated corn stover.

With additional funding ($2 million) from the Ohio Department of Development Third Frontier Program, researchers are working with quasar energy group to commercialize this technology. A wide variety of feedstocks are being tested in the lab to evaluate their biogas potential. The optimal operating conditions will be used for the design and operation of the pilot-scale facility to be built in Zanesville, Ohio by quasar energy group.

Efficacy of Injected Disinfectants in Killing Zoonotic Pathogens and Zoospores of Plant Pathogens in Irrigation Water

Sally A. Miller, Plant Pathology
Jeffrey T. LeJeune, Food Animal Health Research Program

Food safety has become an extremely important factor in fresh vegetable production in recent years. From 1990–2004, vegetables accounted for 51% of produce-related outbreaks. Produce contamination with the zoonotic bacteria *Escherichia coli* or *Salmonella* sp., while rare, has resulted in significant losses system-wide due to product recalls and concerns of consumers for safety. Recent recalls of spinach, tomatoes, and jalapeno peppers are good examples. Contamination of vegetables by zoonotic pathogens can occur during pre- and post-harvest activities. Growers are beginning to invest in injector systems to deliver disinfectants into irrigation water in order to reduce or eliminate harmful phytopathogens and zoonotic pathogens that may be present in surface water. The potential for contamination of surface water with these pathogens from various sources (animal droppings, weeds, soil, and crops) can be significant. In fact, the probability of surface water contamination with plant pathogenic water molds such as *Phytopthora capsici* in an intensive vegetable production area is likely to be much higher than the probability of contamination with a zoonotic pathogen. In this study, investigators determined the relative efficacy of commercial chlorine dioxide or chlorine gas injection systems for irrigation water in killing zoonotic pathogens and plant pathogenic water molds.

To determine if *Phytophthora capsici* and other *Phytophthora* spp. were present in surface irrigation water in an Ohio vegetable production area, we used floating traps containing fresh cucumber as bait in three irrigation ditches and two ponds. Cucumbers were replaced every 3–7 days throughout the growing season. Water temperature and pH were determined at each sampling. Recovered cucumbers were checked for *Phytophthora* infection microscopically and by Polymerase Chain Reaction assay (PCR) using *Phytopthora* genus-specific and *P. capsici*-specific primers. *Phytophthora* was detected in ponds and ditches. Average water temperatures ranged from 20.4°C–23.6°C in the ponds and 20.0°C–24.3°C in the ditches. The pH of the water in the two ponds tested was lower (range 6.8–7.5) than in the ditches (7.4–8.0).

Sally A. Miller

Water was collected in large pails at several points in the chlorine dioxide and chlorine gas treatment systems for irrigation water: non-treated (ditch), after chlorine injection, after sand filtration, and at the emitters. Free chlorine and chlorine dioxide were measured at each sampling point for the chlorine gas and chlorine dioxide treatments, respectively. Cucumbers were placed in the pails as bait for *Phytophthora* spp. Baiting studies were inconclusive, but neither treatment eliminated *Phytophthora* from irrigation water. While chlorine gas was generally more effective than chlorine dioxide at the concentrations tested in reducing coliforms and generic *E. coli* from surface irrigation water, results were variable.

Additional research is underway to determine, under controlled laboratory conditions, chlorine requirements (ppm) to reduce numbers of *Phytophthora* propagules (zoospores, sporangia, mycelia) in water. The water treatment systems as currently engineered (treatment with chlorine prior to sand filtration) may not maintain chlorine concentrations high enough to consistently reduce populations of *Phytophthora* spp., generic *E. coli*, or coliform bacteria. If surface water continues to be used for fresh produce irrigation, re-engineering of the injector systems may be necessary. At a minimum, the efficacy of such systems should be determined before farmers make significant investments in the technology.

Commercial Potential of Replacement of Plant (Gluten) Protein with Algae Protein for Growth Enhancement of Fish

Konrad Dabrowski, School of Environment and Natural Resources

In 2007, U.S. Congress passed the Energy Independence and Security Act, mandating nearly a five-fold increase in U.S. ethanol production by 2022. Nearly half of that is supposed to come from corn whereas algae production appears to be a much more environmentally sustainable crop. Advancement in the biofuel industry is estimated to have a direct U.S. economic output of $5.5 billion in 2012, and feedstock production will become a significant part of this new industry. Therefore, in addition to algae use in human nutrition, microalgae are increasingly incorporated to animal feeds, fish diets (particularly as fish meal), and plant protein replacement.

Microalgae and amino acid profiles have a high-protein content, making them highly valuable components of fish diets. Second, microalgae are carriers of high-value molecules, which have yet to be discovered. It has been demonstrated that microalgae produce numerous antioxidant and antimicrobial compounds that still need to be screened for their activity and utility in fish diet formulations. However, it has been well documented that there are significant differences in acceptability, digestibility, and bioavailability of various algae species when included in diet formulations for fish. Therefore, finding the compromise between the utility of microalgae for biodiesel production in photobioreactors and ponds and their utility in wastewater treatment and animal feedstuff will be a challenge.

Konrad Dabrowski

Evaluation of alternative protein sources for fish meal and plant protein concentrates in fish diets is critical to economic benefits of the aquaculture industry as well as to public perception of aquaculture as depleting or protecting ocean resources. Introduction of algae as the protein source, feed attractant, and source of antibacterial compounds might further increase aquaculture competitiveness in human food production.

The preliminary results regarding the use of microalgae produced for biofuels in diet formulations used to feed Nile tilapia (*Oreochromis niloticus*) indicate that microalgae positively affect food consumption and fish growth. Replacing up to 50% of dietary corn gluten meal protein with microalgae was found to significantly enhance fish growth. Microalgae were also a source of minerals. Differences in concentration of individual minerals in the whole fish body were significant and might have affected growth when diets contained more than 75% of plant protein replaced by microalgae.

Researchers bred tilapia (*Oreochromis niloticus*) in the laboratory and collected juveniles from brooding females. The fish were maintained, essentially, without feeding until commencement of the feeding trial.

The results indicate that the growth rate was significantly improved when up to 50% of the plant protein was replaced at an even faster rate than fish that were fed the control diet. In many respects, the mineral levels in the bodies of these fish are in excess of the minerals reported thus far in fish feeding studies, and the issue requires separate attention in order to include possible waterborne and foodborne toxic effects as well as possible dietary remedies to counteract toxic elements accumulation.

The results of this project will be directly transferable to tilapia producers and feed mills in the North Central Region via research reports, journal publications, presentations at selected aquaculture meetings, and face-to-face meetings with feed manufacturers. The end products will play a significant role in the development of the U.S. and Ohio aquaculture industry. Due to the wide availability of algae meal and algae oil from biodiesel outdoor mass production proposed by Independence Bio-Products Development, LLC, there is tremendous potential for the utilization of algae in the aquaculture industry that could prove beneficial for the fish and profitable to producers by enhancing feed acceptance and growth rate. Any improvements of fish growth that result from the use of microalgae in fish diets would be immensely beneficial to U.S. fish farmers due to intense competition from fish producers in other countries.

Limiting Antibiotic Resistance Microflora in Dairy Fermentation

Hua Wang, Food Science and Technology
Valente B. Alvarez, Food Science and Technology

Hua Wang

The rapid emergence of antibiotic-resistant (ART) pathogens is a major threat to public health. Recently, it was found that ART bacteria are prevalent in many ready-to-eat products. Particularly, a large antibiotic resistance (AR) gene pool was found in commercial cheese products, and characterized AR gene carriers include several lactic acid bacteria commonly used as fermentation starter cultures.

The objective of this proposed study was to work with the U.S. dairy industry to identify key processing conditions to minimize the emergence of ART bacteria in dairy fermentation. This line of research is of great importance to food safety and public health. The research outcomes will benefit the dairy fermentation industry, and the impact is worldwide.

At The Ohio State University's dairy pilot plant, researchers created different cheeses with different combinations of starter cultures and processing conditions. Investigators also visited commercial cheese-manufacturing facilities. They examined the tetracycline-resistant bacteria isolated from various samples collected during cheese-making, and they analyzed the resistance gene profiles of the ART isolates.

Researchers found that antibiotic contamination varied among products made at The Ohio State University dairy pilot plant, which was correlated to processing management. Commercial starter cultures were free of the tetracycline genes examined. Raw milk was highly contaminated with ART bacteria, but pasteurization significantly reduced the amount of the ART bacteria. Most of the samples from the commercial production facility contained low-level contamination of ART bacteria, with the identified AR gene carriers being non-starter lactic acid bacteria and other organisms.

Suggestions on minimizing ART bacterial contamination by proper processing control have been delivered to the dairy industry. In addition, as a result of the studies and the collaborative efforts from the U.S. dairy industry and starter culture companies, those starter cultures with AR genes—including the bifidobacterium-containing tetracycline resistance gene previously supplemented to yogurt product all over the world—were removed from the market. The prevalence of AR in name-brand cheese products was reduced significantly within the past couple of years. Scientists identified additional critical control points in dairy fermentation and are recommending them to the dairy industry.

In addition, results from SEEDS support directly lead to a national initiative and related efforts to change the agriculture policy in AR and food safety. A synthesized expert report/recommendation was developed and submitted to the USDA Cooperative State Research, Education, and Extension Service and the USDA Agricultural Research Service for future implementation. Researchers will continue leading this important food safety and public health research initiative in the U.S. Future plans include expanding the research coverage to include other food commodities and working with scientists from other countries to ensure global food safety.

Optimization of Natural Fibers Processing Equipment for Plastics Reinforcement

Harold M. Keener, Food, Agricultural, and Biological Engineering
Robert C. Hansen, Food, Agricultural, and Biological Engineering
Michael H. Klingman, Food, Agricultural, and Biological Engineering

Bast fibers have the potential to replace glass fibers for reinforcement of plastics and composites in a variety of industrial applications. These fibers have similar strength characteristics and have the added benefits of being lighter, renewable, safer, and less costly. Current technologies that incorporate bast fibers are inefficient and expensive. However, Natural Fibers Composite Corporation (NFCC) has developed a novel technology that fibrillates the fiber bundles into finer particles, thus overcoming these challenges. While private and state funding has been secured to implement this technology, additional research is needed to determine optimal operational parameters.

The goal of this project is to optimize the performance and maximize the efficiency of fiber processing equipment with regard to quality, consistency, and production rate of the fibrillation process for a variety of bast fibers such as kenaf, jute, and flax.

Fiber processing equipment (FPE) was purchased, delivered, and successfully installed and operated in an agricultural engineering lab at the Ohio Agricultural Research and Development Center. The FPE was successfully redesigned and equipped with a new high-capacity Downflo Oval dust collection system after the dust suppression system supplied by the manufacturer was found to be inadequate. The original unit did not have the capacity required to handle the quantity of dust generated by the fibrillation process. After the upgrades, several hundred pounds of fibrillated kenaf fiber were produced and delivered to NFCC facilities during the first year (2008) in preparation for the formation of reinforced plastic pellets, which result from compounding the fiber with plastic resin.

Fibril samples were successfully evaluated using optical microscopy and image analysis software to determine the cumulative size distribution of fibril cross-sectional areas. Once fiber fitness is determined, light microscopy images along with fiber length and diameter analyses from ImageJ software can be used to monitor fibril quality. This methodology will ultimately provide a procedure for measuring and evaluating the quality of the fibrillated product during the manufacturing process.

(left to right) Robert Hansen, Michael Klingman, Harold Keener

A two-level, six-factor, fractional-factorial experiment in sixteen runs was designed as part of optimizing the fibrillation process. Example factors to be evaluated included type of fiber, fiber length, fiber moisture content, screen opening size, throughput rate, and relative humidity. Preliminary results suggest that increased throughput may lead to smaller particle cross-sectional area distributions when using different screen sizes, as would be expected. If these results can be confirmed in future test runs, then significant increases in throughput rate will be possible. Concurrently, increased production per unit of energy (kg/kwh) would also be expected.

With the global market for reinforced plastics currently at approximately $25 billion and growing by 5% per year, there are significant opportunities for replacing glass fibers with renewable bast fibers. This research will benefit Ohio's agricultural sector by providing a market for new crops as well as the industrial sector as a pilot-scale manufacturing plant is being developed in Wooster, Ohio.

Enhancing Seed Health and Vigor in Tomatoes

Sally A. Miller, Plant Pathology
Mark A. Bennett, Horticulture and Crop Science
Brian B. McSpadden Gardener, Plant Pathology

Bacterial canker is one of the most important diseases affecting tomato production worldwide. Economic losses due to reduced productivity of infected plants, removal of symptomatic plants, and costs of additional sanitation practices are substantial. The pathogen *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) is seedborne and may be present as a surface contaminant or under the seed coat. Although propagation practices—especially grafting and pinching—can spread the pathogen, its initial means of entry into a crop is most likely Cmm-infested seed. Therefore, seed must be the first point of focus in developing an integrated program to manage this disease. Although effective seed treatments have been identified in the past, most adversely affect seed and seedling vigor.

The objective of this study was to evaluate the effectiveness of seed treatments in freeing seed from the bacterial canker pathogen without reducing seed and seedling vigor. Tomato growers who adopt this treatment can be assured of starting off the production cycle with healthy, vigorous seed and seedlings.

Tomato seeds naturally infected with the bacterial canker pathogen were treated with various sanitizing treatments. The seeds were then cultured to determine if any bacteria remained. Others were planted in the greenhouse under typical conditions for tomato seedling production, and seedlings were checked for bacterial canker symptoms for up to 6 weeks. Seedlings without symptoms were selected at random, and any resident bacteria were washed from the tissues and cultured to identify epiphytic Cmm (living on the seedlings but not causing disease) populations.

The disinfectants HCl (hydrochloric acid), NaClO (sodium hypochlorite, the main ingredient in bleach), Kleengrow, and Virkon were most effective in killing Cmm both on and in seed. Greenhouse testing of disease incidence and Cmm populations were consistent and confirmed that these seed treatments reduced the disease incidence and Cmm population on seedlings. In addition, these disinfectants did not influence seedling vigor up to 12 months after treatment compared to the untreated control.

Scientists plan to provide this information to the seed industry and to vegetable growers nationally and internationally. Investigators also plan to continue studies on the biology of Cmm infection of seed and seedlings to better understand how bacterial canker epidemics start and how to prevent them. Scientists will continue the seed vigor analysis with a final reading 18 months after treatment.

Sally A. Miller

An Economic Assessment of the Emerging Renewable Polymer Cluster in Ohio

Thomas Sporleder, Agricultural, Environmental, and Development Economics
Florian Diekmann, University Libraries

The economic impact of the chemical and polymer cluster in Ohio in terms of output, gross state product, income, and employment is analyzed in this applied economic research. The objective was to quantify the economic contribution of the polymer industrial supply chain in the context of value-added industries. An input-output model was constructed that captures inter-industry linkages among various polymer sectors and industries. The economic measures of importance are output, gross domestic product, income, and employment.

The chemical and polymer cluster positively affects the state's economy, representing 5.5% of Ohio's gross state product, 9% of its output, over 6% of its income, and 3% of the state's employment. The chemical and polymer cluster represents 2,729 establishments in Ohio. Estimates are that the renewable polymer industry will represent more than a 12% share of Ohio's polymer sector in the year 2020 in terms of gross state product and employment. Thus, substantial growth is forecast. Nationally, for the same year, the input-output analysis indicated that chemical and polymer cluster output accounted for about 5% of total gross domestic product. U.S. gross domestic product (GDP) was $13.8 trillion, with approximately $679.9 billion accounted for by the polymer industrial supply chain. The chemical and polymer industrial supply chain cluster contributes $4.92 of each $100 of U.S. GDP.

One distribution and four manufacturing sectors were defined as the chemical and polymer industrial supply chain. These include 1) petroleum and natural gas extraction, 2) chemicals manufacturing, 3) polymer manufacturing, 4) mold and equipment manufacturing related to polymer production, and 5) chemical and polymer wholesale distribution. The polymer manufacturing sector was defined to include five subsectors: 1) coated and laminated packaging, paper, and plastics film; 2) plastics material and resin, synthetic rubber, and organic fibers; 3) paints, coatings, and adhesives; 4) plastic products; and 5) rubber products. The polymer sector contributes about 15% of the total GDP contribution accounted for by the entire industrial supply chain. The largest subsector within the polymer sector is plastics products manufacturing, which accounts for more than half of the total GDP contribution by the polymer sector. Three-fourths of the contribution from the polymer sector comes from plastics products manufacturing and plastics material and resins and synthetic rubber manufacturing.

Thomas Sporleder

Food Applications of a Handheld, Portable Infrared Sensor

Luis Rodriguez-Saona, Food Science and Technology

Luis Rodriguez-Saona

The food processing industry is the largest contributor to Ohio's manufacturing economy, accumulating $5.9 billion per year. On-line, or real-time, spectroscopic methods can provide a valuable window into in-process food manufacturing in order to optimize the production rate, quality, and safety of foods. Advances in infrared spectroscopic instrumentation combined with multivariate data analysis make this technology ideal for the snack food industry. The objective of this research was to develop a real-time methodology for monitoring fortification of whole grain cornmeal and oil quality using a handheld infrared sensor.

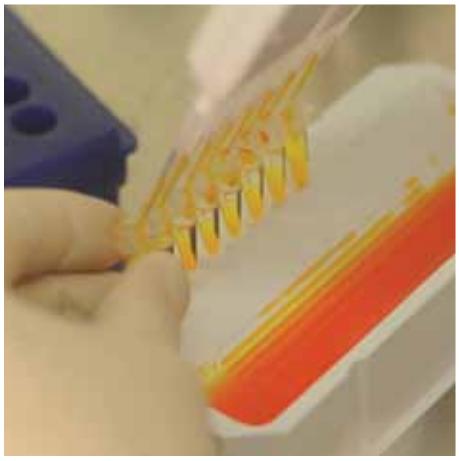
Whole grain cornmeal was fortified with a blend of zinc, iron, vitamin E, and calcium at different levels (0.5–5.5%) and mixed for 30 minutes to ensure homogenous distribution of the fortificants. Real-time infrared analysis was achieved by pressing an aliquot (0.1g) onto an ATR diamond crystal of a portable handheld spectrometer, and spectra were collected. Pattern recognition analysis was used to examine the data collected for monitoring distribution uniformity. The model predicted the level of fortification and was able to cluster the samples into classes corresponding to three different fortification levels. An independent validation set showed improved performance statistics using the handheld infrared spectrometer rather than a bench-top infrared unit with a standard error of predication.

A portable, battery-operated handheld ATR-IR spectrometer allowed for the reliable determination of mineral fortification levels in whole grain cornmeal, monitoring oil quality and quantitation of trans-fat content. This technique provides for the fast analysis of food components with minimal personnel training, simple data acquisition, and immediate predictions. The handheld device provided greater versatility, ruggedness, and portability as a real-time infrared sensor for monitoring quality assurance, providing the industry with a tool for timely correction measures during manufacture.

Next steps will include implementation of these infrared sensors for real-time and field-based measurements for surveying processes and products, controlling the raw material stream, sensing the final product quality, and providing nutritional and health information. The end product would be a simple and automated system that would provide Ohio food manufacturers tools for early corrective actions that will ultimately save time and money while establishing a uniform quality.

Seed Grant Competition

The Seed Grant Competition is designed to encourage new and innovative research and to generate the preliminary data needed for successful application to competitive extramural funding sources. Seed Grants are supported at a maximum level of $50,000.



Using Stable Isotope Analysis to Link Seasonal Events in Migratory Landbirds

Paul G. Rodewald, School of Environment and Natural Resources

Songbirds that migrate between North America and the tropics have shown recent population declines, which have been attributed to events occurring either on breeding grounds (nest predation), wintering grounds (tropical deforestation), or during migration. Understanding geographical and ecological linkages between breeding and non-breeding areas is important because habitat conditions experienced in one area may carry over to influence subsequent events of a bird's annual cycle. For example, a bird that spends the winter in a poor quality habitat may attain unfavorable body condition and ultimately arrive in breeding areas late and in less than desirable condition, which ultimately will influence the bird's ability to reproduce.

Stable isotope analyses have recently been used to study long-distance migratory movements and nutritional origin in animals. This approach is based on the fact that isotopic signatures in an animal's environment are reflected in its tissues and that this information varies geographically. For example, isotopic signatures of stable hydrogen in animal tissues can be used to link migratory individuals to the geographic origin where the tissue was grown. Stable isotope analyses of animal tissues also can identify the type of food or habitat used by a feeding animal.

The first of three broad objectives in this study involved carry over effects in the annual cycle of migratory landbirds. Researchers examined relationships between the quality of winter habitat used by Magnolia Warblers captured during spring migration in northwest Ohio. Investigators examined stable carbon isotopes in feathers grown in the tropics, where the birds winter. Researchers hypothesized that drier habitats would advance infection by blood parasites, delay migration, and lower energetic condition and amount of molt completed. The captured birds wintered over a range of dry to moist habitats, but there was no detected relationship between habitat moisture and migration timing, energetic condition at capture, number of molted wing coverts, plumage coloration, or blood parasite infection rates. The results suggest that habitat moisture may not be the primary factor affecting winter habitat quality or that individuals examined did not experience carry over effects. There is a need to better understand interactions among phases in the annual cycle of migratory songbirds to develop plans for their conservation.

The second objective involved the use of stable-hydrogen analysis to identify breeding locations of migratory songbirds that stop to feed and rest in northwest Ohio. This aspect of the study focused on the Gray-cheeked Thrush, the only songbird to migrate across the Bering Strait to Russia for breeding. As a result, this species had been identified as a potential carrier of the high pathogenic avian influenza (HPAI) strain of H5N1. Results indicated that some thrushes passing through Ohio may breed in regions where HPAI occurs, but the large majority seem to originate from elsewhere in the boreal forest of Canada. Therefore, this songbird poses little threat for the transmission of HPAI H5N1.

The third objective was to examine how refueling by spring migrant landbirds can affect the acquisition of quality territories and mates. Spring migrants often encounter adverse weather and low availability of insect prey, but insects such as midges that hatch from aquatic habitats may provide an important food resource. Scientists detected no relationship between diet and energetic status for any species. Researchers suggest that conservation and restoration of shoreline and inland forest patches within landscapes containing wetlands can promote refueling by migrant landbirds.

Additional support for this research was received by the Ohio Division of Wildlife to conduct the study. The continued use of stable-isotope analysis to examine seasonal carry over in migratory birds is planned because this is an important tool for determining how and when declining species are most negatively affected by annual events.

Paul G. Rodewald

The Role of Adenosine Kinase in Metabolic Defense Signaling

David Bisaro, Molecular Genetics

This project was conducted to better understand the nature of an antiviral defense response mediated by SNF1-related kinase (SnRK1). SnRK1 in plants belongs to a highly conserved family that includes SNF1 in yeast and AMP-activated protein kinase (AMPK) in animals. These kinases are involved in the regulation of cellular energy homeostasis and play important roles in many biological processes in addition to pathogen defense—including maintenance of normal metabolism and growth—and cancer.

SNF1/AMPK/SnRK1 kinases are involved in the regulation of cellular energy homeostasis and in response to stresses that deplete ATP, their role is to inhibit energy-consuming biosynthetic pathways and promote catabolism. Cellular energy stress is sensed by increased AMP:ATP ratios. When AMP levels are high, the SNF1/AMPK/SnRK1 kinases are activated, in part by allosteric effects mediated by AMP itself. In previous studies, scientists showed that SnRK1 is a component of antiviral defenses and that geminivirus pathogenicity proteins interact with and inhibit SnRK1. In addition, the same viral proteins interact with and inhibit adenosine kinase (ADK), which phosphorylates adenosine to generate AMP. This suggested a relationship between SnRK1 and ADK, which researchers investigated in this project using a combination of in vitro and in vivo approaches to test for physical and functional interaction.

Investigators found that SnRK1 and ADK physically associate in complexes that accumulate in the cytoplasm and that SnRK1 stimulates ADK in vitro by a novel, non-enzymatic mechanism that involves direct and stable contact between these proteins. Further, experiments with transgenic plants having altered SnRK1 or ADK activity have provided strong evidence for in vivo linkage of the two kinases. In summary, this study establishes the existence of SnRK1-ADK complexes that likely play important roles in energy homeostasis and cellular responses to biotic and abiotic stress.

A manuscript describing this work has been prepared, and submission is expected in January 2011. A grant proposal to further investigate the nature of SnRK1-ADK complexes and their functional significance is also in preparation for submission to the National Science Foundation in July 2011.

David Bisaro

Determination of the Glycemic, Insulin, and Satiety Index of a Soy Pretzel in Healthy Individuals

Yael Vodovotz, Food Science and Technology

Obesity and diabetes often occur concurrently, and both conditions are major contributors to the chronic disease burden that faces the nation as a whole. There are important linkages between specific foods in a diet and factors that promote these chronic diseases. Food properties such as the glycemic index (GI), energy density, and macronutrient content are important factors in the prevention and/or management of diabetes and in weight control. For example, low-GI foods may be used to reduce postprandial glycemia, and the frequent use of these foods improves overall blood glucose control in diabetes. Low-GI foods may also be helpful in weight control by either promoting satiety or by enhancing fat oxidation at the expense of carbohydrate oxidation. Further, foods that are low in energy density (energy per unit of eight, e.g., kcal/g), high in dietary fiber, and/or high in protein may improve satiety, thus reducing a person's food intake. Soy foods are of particular interest because they have the combined attributes of being low on the GI, low in energy density, high in dietary fiber, and high in protein content.

The long-term goal of this project's investigators was to develop new dietary strategies for the maintenance of healthy blood glucose levels, especially with regard to diabetes and pre-diabetes. The overall objective of this research was to determine how the consumption of a soy-enhanced soft pretzel affects postprandial glucose and insulin responses, and to determine if the soy pretzel can increase satiety relative to a conventional pretzel. The central hypothesis was that a soy pretzel would cause a lower blood glucose and insulin response relative to a conventional pretzel, and that, further, it would promote short-term satiety.

Short-term satiety and sensory acceptability were high for both wheat and soy pretzels, but no statistical difference was found. These results indicate that the soy pretzel was as acceptable as its wheat counterpart and that the individuals consuming the products felt the same degree of fullness from both. The GI was found to be significantly lower for the soy pretzel as compared to the wheat pretzel, although insulin values were not statistically different for the two.

Yael Vodovotz

This preliminary clinical trial demonstrated great potential for transforming snack foods into health-promoting alternatives. Soy-enriched pretzels will be further studied in an attempt to lower their GI and optimize their nutritional quality and consumer acceptability. The addition of healthy fats such as olive oil will not only improve mouthfeel but will potentially improve the texture of a soy pretzel when it is microwaved from a frozen state. Additionally, in vitro digestion will be used to analyze the type of fat added for its impact on the bioavailability of phytochemicals (such as isoflavones) found in soy.

Genome Scanning and the Genetics of Adaptation within the Apple Maggot Fly, *Rhagoletis Pomonella*

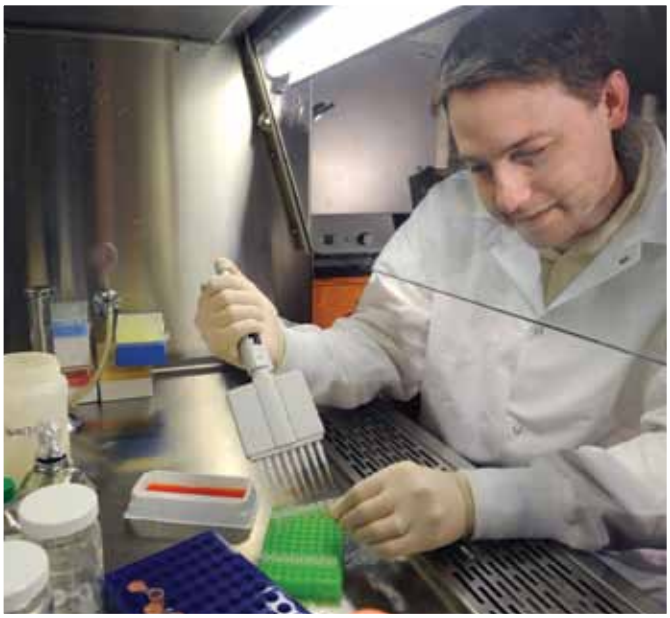
Andrew Michel, Entomology

Understanding the genetic basis for adaptation and divergence of natural populations is an important goal of ecological and evolutionary biology. This is perhaps most evident in current natural systems that are experiencing rapid and large-scale environmental change, much of which is human-mediated. As some organisms struggle to adapt, others thrive in these environments, diversifying and expanding their populations. Identifying the genetic and environmental factors responsible for facilitating adaptation—and whether these factors can be managed—may help to promote threatened species and control pests. Insect pests represent good examples of natural populations adapting to recent human-induced environmental changes, and research into pest adaptation can be extrapolated for further understanding of insect pest genetics and biology. In addition, because insects cause significant damage to agricultural commodities, understanding how insects become pests through the interplay between genetics and environment can improve pest diagnostics and management strategies.

Scientists proposed to investigate insect pest adaptation using *Rhagoletis pomonella* as a model. This species, commonly known as the apple maggot fly, is a significant pest of apple production. The apple maggot fly naturally infests fruit of the hawthorn tree. Both the apple maggot and hawthorns are native to the U.S. After the purposeful introduction of apples 200 years ago, the apple maggot adapted and underwent a host shift to infest this newly cultivated crop. This pest causes millions of dollars in damage, and because most *Rhagoletis* species look alike, it is also difficult to manage with trapping. The goal of this research was to use a molecular marker genome-scan approach to compare non-pest *R. pomonella* populations (hawthorn) to pest *R. pomonella* populations (apple) and to uncover areas of the genome that may be responsible for the host shift.

Genome scanning involves surveying a large number of molecular markers in populations differing in behavioral traits. If the behavioral difference is caused by a genetic factor, then using such a large number of markers increases the likelihood of finding ones that are involved in adaptation or

Andrew Michel



are linked to genes of adaptation. Various marker systems have been used in the apple maggot fly, which suggests that genetic differentiation between the apple and hawthorn flies is widespread. However, because these markers survey only a limited region of the genome, what is needed is a marker system that looks at hundreds of markers. Amplified fragment length polymorphisms (AFLPs) have seen a resurgence in popularity.

Using AFLPs, researchers identified 374 markers in the collections. Scientists simulated what the expected difference among populations would be, and the overlaid the observed results with the expected distribution. Researchers identified a total of 10 markers as significantly different.

Investigators were able to uncover markers that were significantly different among the hawthorn and apple populations, and therefore, potential diagnostic molecular markers will now be validated in future populations. Previous evidence with the apple maggot fly has suggested the presence of chromosomal inversions, where whole segments of a chromosome are flipped, resulting in changes of the linear order of genes. This could be affecting the analysis, where each population has a different linear order; markers linked to genes in one population may not be the same in other populations. Future studies will involve in silico comparisons to recently developed molecular resources. Large amounts of DNA sequence information of the apple maggot have been generated using new sequencing technologies. While this data is just being analyzed, an additional test would be to mine this data to see if genes are found in fragments predicted by the AFLP markers.

Development of a Consolidated Bioprocess for Converting Lignocellulosic Biomass to Biofuel and Biobased Products

Yebo Li, Food, Agricultural, and Biological Engineering

Yebo Li

Concerns over energy security and global climate change have inspired worldwide research and the development of biofuel to replace fossil fuels. The *Annual Report to Congress on the Biomass Research and Development Initiative for FY 2006* states that by 2030, biofuels will replace roughly 20% of the total market share in the U.S., which is equivalent to 51 billion gallons of gasoline per year. Currently, ethanol in the U.S. is primarily produced from sugar- or starch-based crops (e.g., sugar beet, sugar cane, or corn), which competes with food and feed needs. Lignocellulosic biomass—which is sufficiently abundant and widespread and comes mainly from agricultural residues and forestry sources—is the most promising alternative source for the production of ethanol and other biofuels and bioproducts.

The complex structure of native lignocellulosic biomass, however, is highly resistant to enzymatic hydrolysis, resulting in low cellulose conversion. Therefore, a pretreatment process is needed to reduce the biomass recalcitrance by breaking lignin seals and disrupting the crystalline structure of cellulose for improving enzymatic hydrolysis. Compared to the current leading pretreatment processes, microbial pretreatment of lignocellulosic biomass with white rot fungi is considered to be an environmentally friendly process that possesses the following advantages: no use of severe chemicals and elevated temperature, no generated waste stream, and reduced or no inhibitor to fermentation. Several weeks to months are generally needed to obtain a high degree of lignin degradation with microbial pretreatment. However, when the microbial pretreatment is conducted concurrently with on-farm wet storage, the pretreatment time is no longer an issue. Compared to traditional ensilage methods, applying fungal pretreatment to wet storage has the potential to substantially improve the susceptibility of corn stover to enzymatic hydrolysis.

The objectives of this study were to develop cost-effective pretreatment methods for the production of biofuels and bioproducts from lignocellulosic biomass. Investigators applied different methods (microbial, hydrothermal, and alkali) for the pretreatment of corn stover and compared their performance in terms of sugar yield through enzymatic hydrolysis tests and ethanol and lactic acid yields via fermentation tests. These results indicated that high glucose yield can be obtained through hydrothermal pretreatment, but high levels of inhibitors that are toxic to the fermentation process are also generated.

Researchers also investigated the microbial pretreatment of corn stover with *Ceriporiopsis subvermispora*, a white rot fungus. The effects of particle size, moisture content, pretreatment time, and temperature on lignin degradation and enzymatic hydrolysis yield were studied. The results showed that *C. subvermispora* selectively degraded lignin up to 32% with a limited cellulose loss of less than 6% during a pretreatment. The overall glucose yield of *C. subvermispora*-treated corn stover reached about 70%. Compared to current thermochemical methods (which use high pressure and high temperature), the microbial pretreatment process developed in this study can be conducted at ambient temperature without any chemical additions.

To overcome the long pretreatment time issue, researchers will continue investigating the feasibility of on-farm concurrent wet storage and microbial pretreatment with funding from the U.S. Department of Energy. Researchers will also develop an integrated process for the production of fuel ethanol and polymers from lignocellulosic biomass with funding from the USDA. Investigators will also work with U.S. ethanol industry partners such as POET (who has expressed interest) to scale up and commercialize the developed concurrent wet storage and microbial pretreatment technology.

Characterization of Chicken T Regulatory Cells

Ramesh Selvaraj, Animal Sciences

During infections in chickens, invading pathogens are killed by what is currently known as activated T cells. Once the infection subsides, activated T cells are suppressed by a special subset of T cells, namely T regulatory cells (Tregs). Treg-mediated suppression is one mechanism through which the immune system protects the host from excessive responses and maintains self-tolerance. Chicken T regulatory cells were yet to be identified at the time of this research.

This research sought to answer these six questions:

- Do Tregs exist in chicken?
- Can iTregs be induced in chicken?
- Do Tregs suppress nave T cell proliferation in vitro?
- What is the cytokine production profile of chicken Tregs?
- What other markers are expressed by chicken Tregs?
- Can Tregs induce T cell energy in vivo?

Researchers have shown successfully that chicken Tregs exist in chickens. Scientists induced iTregs in chickens. Results showed that both Tregs and iTregs suppress nave cell proliferation in chickens. Investigators hope to study how these chicken T regulatory cells will be applied to modify inflammation in vivo in chickens.

Ramesh Selvaraj

Development of Chemical Markers as Indicators of Process Non-Uniformity During Pressure-Assisted Thermal Processing of Low-Acid, Shelf-Stable Foods

V.M. Balasubramaniam, Food Science and Technology

Pressure-assisted thermal processing (PATP) is a paradigm-shifting technology that is on-trend with consumer interests on minimally processed low-acid canned foods that are preserved with few or no preservatives. The unique advantages of this technology include a rapid increase in the temperature of treated food samples and the expansion cooling of products upon depressurization. Consequently, PATP better preserves product attributes such as color, flavor, texture, and nutritional values when compared to conventional thermal processing. Depending upon the severity of pressure-heat combination used, the technology enables pasteurization and sterilization of foods. Guacamole, tomato salsa, sliced meats, ham, poultry products, oysters, fruit juices, and smoothies are some of the examples of value-added commercial pasteurized products currently available in the market. High-pressure treatment is physical in nature (similar to the pressure cooker used at home, but at significantly higher pressures) and it is readily accepted by the consumers.

Pressure-accelerated, thermally processed, shelf-stable products such as low-acid soups are not commercially available yet. Obtaining such products is a topic of current research. One of the key research questions is, how uniform is the combined pressure-heat treatment? Similar to traditional thermal processing, identification of the least-processed volume during treatment will help ensure safety of processed foods. Further development of mathematical models to describe combined pressure-heat treatment on microorganisms and food products can help processors optimize the process.

Food is a complex mixture of carbohydrates, proteins, fats, vitamins, minerals, and water. At high temperatures (>100°C), certain carbohydrates and proteins react to form a number of compounds. A marker known as the M-2 marker was successfully used as an indirect indicator of least-treated zones in advanced thermal processes such as aseptic processing, microwave sterilization, and ohmic heating. The results suggest that the M-2 marker may not be a suitable for evaluating pressure-heat uniformity during PATP. Our plan is to continue to screen additional carbohydrate-protein compounds that may be suitable candidates for this purpose.

A mathematical model was successfully developed and validated for estimating accumulated microbial lethality of spores undergoing combined pressure-heat treatment. The model was experimentally validated under several process scenarios using a pilot-scale high-pressure food processor. The model computed accumulated lethality, and the log reduction values were in reasonable agreement with experimental data.

The developed model can be a useful tool to examine the effect of combined pressure-thermal treatment on bacterial spore lethality and to assess PATP microbial safety. This will help the food industry demonstrate the microbial safety of pressure-sterilized, low-acid foods.

V.M. Balasubramaniam

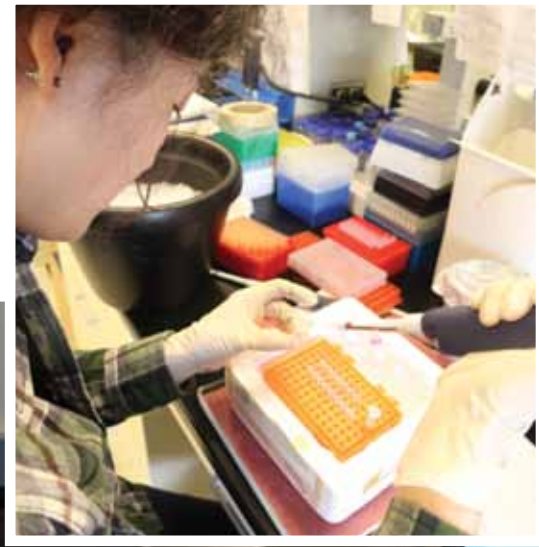
Development of a Triplex Microsphere-Based Assay for the Detection and Differentiation of H5 and H7 Hemagglutinin Subtypes of Influenza Virus

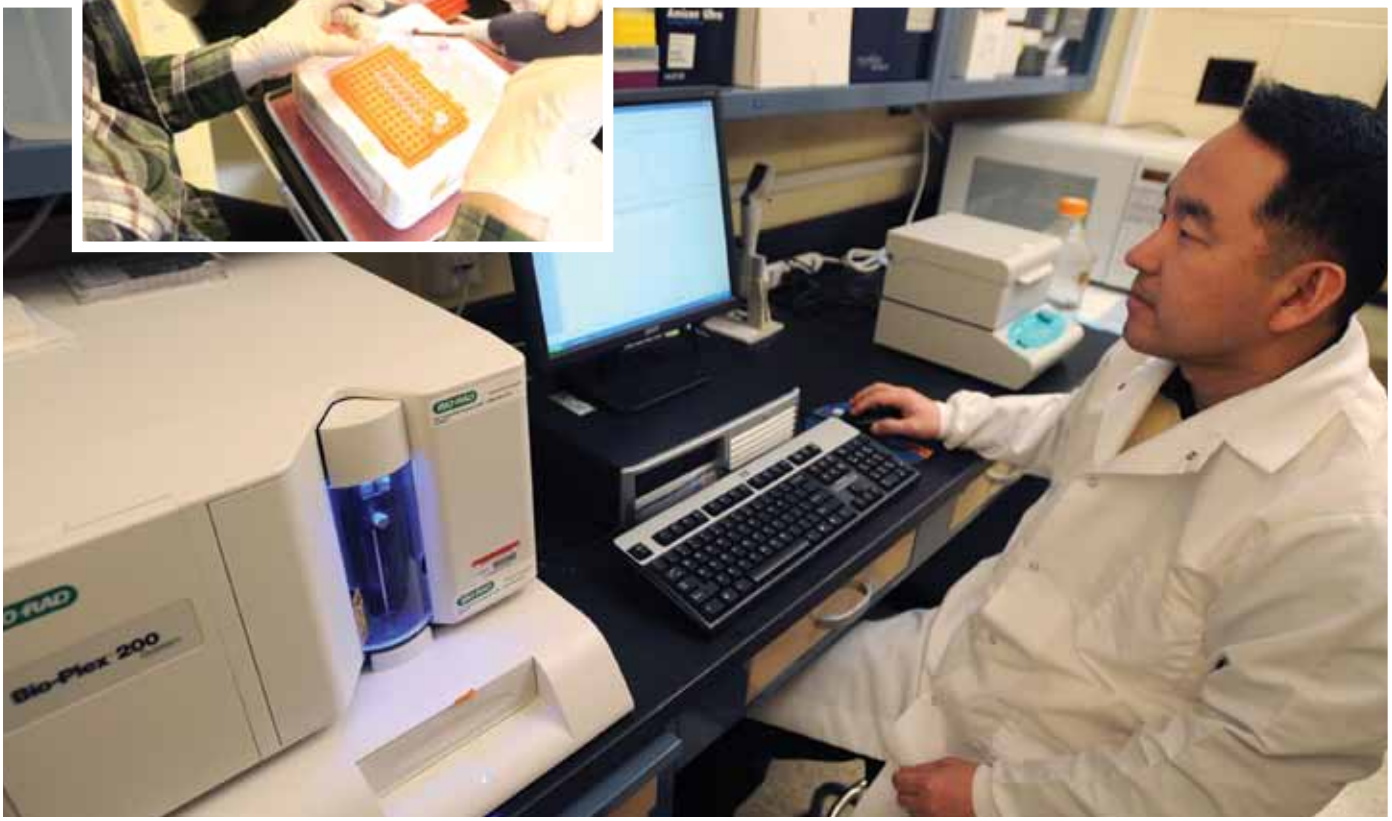
Chang W. Lee, Food Animal Health Research Program

Rapid and precise detection of influenza virus is essential for controlling further spread into domestic animals and for preventing possible transmission to humans. Real-time RT-PCR (RRT-PCR) has been developed as a rapid and economically feasible diagnostic alternative to conventional methods. It has shortened the time of detection from 1–2 weeks to less than a couple of days, and labor has been markedly reduced as a result. However, the main disadvantages of using RRT-PCR are its high-assay cost and the limitation in multiplexing, which further increases the cost as well as the assay time.

In this study, scientists established 19-plex assay, which can detect all type A influenza viruses and differentiate 16 different HA or lineage of avian influenza viruses. In addition, the assay can detect pandemic H1N1 strains. The 19-plex assay showed high specificity with minimal cross reactivity among different subtypes and similar MFI value as observed with the monoplex assay. Overall, in addition to multiplex capacity, this system shows good reproducibility and does not require an RNA extraction step. Another bonus is the fact that swabs, cell culture supernatant, and fluid samples can be directly applied for the assay.

Since the test has been bench-validated, researchers will focus on testing and validation of clinical and surveillance samples. In addition, investigators will further extend the application of this technology to detect different pathogens including food safety-related pathogens. Prompt identification and characterization of influenza viruses and other pathogens from diverse sources in Ohio is essential for early protection of the animal industry and of Ohio citizens.





Chang W. Lee

Towards Better Lignocelluloses to Butanol Conversion

Thaddeus Ezeji, Animal Sciences

As nationwide demand for fuels and chemicals surges, producers of biofuels are increasingly looking beyond corn for main substrates. However, many of the proposed alternatives such as switchgrass and *Miscanthus* appear to be a long way from becoming the substrate of choice for butanol and ethanol production. Yet the truth is, without the development of efficient technology for lignocellulosic biofuels production, renewable liquid fuel will remain elusive and the nation will not be able to displace significant percentages of imported oil with renewable biofuels. The U.S. federal government has supported and funded research in the area of biomass processing and conversion into fuels and chemicals in recent times. This federal initiative may provide a tremendous opportunity for research and development in Ohio, where there is an existing strong agriculture and food processing industry base. Converting agricultural wastes (stalks from corn, soybean, etc.) and readily available forest residues to biofuels and chemicals is the most promising short-term strategy to meet the fuel and chemical needs of Ohio. Energy crops (switchgrass, *Miscanthus*, hybrid poplar, industrial sweet potatoes, etc.), forest residues and wood wastes, and municipal wastes offer long-term strategies and additional opportunities for utilization and conversion to fuels and chemicals.

The total available lignocellulosic biomass including agricultural and forest residues, wood wastes, and energy crops has been estimated to be about 19 million tons in the state of Ohio. This biomass resource has the potential to produce over 1,500 million gallons of acetone-butanol (AB) or ethanol. Efficient production of biofuels and chemicals from the Ohio-based lignocellulosic biomass resource will provide new business opportunities for the biobased industries in Ohio and will stimulate the creation of new jobs in the processing, distribution, and marketing of biofuel and chemicals. For example, The Department of Energy estimates that production of 90 million gallons of ethanol per year from a lignocellulosic source will generate 2,800 direct and indirect jobs. Therefore, investigators estimate that the production of 1,500 million gallons of lignocellulosic butanol or ethanol in Ohio will generate over 45,000 new jobs.

Major limitations of the lignocellulosic biomass conversion to butanol and other value-added products are biomass recalcitrance and the inhibitory effects of degradation

Thaddeus Ezeji

products of lignocellulose hydrolysis. Due to recalcitrance of lignocellulosic biomass, biomass must be pretreated before it can be hydrolyzed into simple sugars. Unfortunately, during pretreatment and hydrolysis, a complex mixture of microbial inhibitors is generated, thereby rendering biomass hydrolysates unfriendly for the growth of fermenting microorganisms. The fermentation of lignocellulosic biomass such as corn stover, wheat straw, switchgrass, etc., to butanol by solventogenic clostridia must be improved for profitable use of these substrates for butanol production and chemicals. To generate inhibitor-tolerant clostridia cells, scientists subjected *Clostridium beijerinckii* 8052 cells to mutagenesis and adaptation using different concentrations of ethyl methanesulfonate and corn stover. Knowledge garnered from this investigation will be used to manipulate candidate gene(s) of *C. beijerinckii* mutant and optimize inhibitor tolerance for the purpose of improving butanol production from lignocellulosic biomass.

It is important to note that during the course of this project, researchers identified an environmentally friendly catalyst that improved substrate utilization by 35%, acetone-butanol production by 65%, and AB productivity by 100% in solventogenic clostridia. These findings have the potential of moving bioproduction of butanol closer to commercialization, and an application for patent has been submitted.

Towards the Cloning of the Tomato FS8.1 Gene Using Differential Expression and Massively Parallel Signature Sequencing (MPSS) Technology

Esther van der Knaap, Horticulture and Crop Science

Shape and size are important selection criteria in fruit and vegetable breeding programs. Fruits that are too small are not economically viable for growers, and fruits that are too round are not conducive to mechanical harvesting. Consumers also associate certain shapes and sizes of produce with familiarity or novelty of different produce. Thus, it is of critical importance that new tomato varieties develop fruit of the appropriate dimensions for a particular market segment. The tomato germplasm consists of many varieties that differ in fruit shape and size. The identification of the genes that control this diversity will enable breeders to select the right combination of genes and alleles that will result in fruit of certain shape and size at an early stage in the breeding cycle.

This study focused on the identification of a gene that controls elongation of the tomato. Prior research demonstrated the importance of this elongation gene, especially in field-grown tomatoes. Knowledge of the gene will enable the employment of a molecular marker and expedite the development of improved breeding lines. The fs8.1 locus maps to a region of the genome that does not recombine, precluding genetic approaches to identify the underlying gene. Therefore, researchers proposed a novel technique aimed at identifying a small set of candidate genes from which we would narrow down the factual FS8.1 gene. Researchers applied state-of-the-art technology, using newly acquired equipment through the Ohio BioProducts Innovation Center and housed at the Molecular and Cellular Imaging Center on the Ohio Agricultural Research and Development Center campus. The identification of FS8.1 will potentially enable breeders to more effectively introgress this region into elite germplasm. In addition, the gene would allow insights into the molecular nature of fruit shape variation in tomato and other important vegetable crops.

This project aimed at identifying candidate genes underlying the fruit shape locus fs8.1. Using the Massively Parallel Signature Sequencing (MPSS) technique—which is now called Sequencing by Synthesis or SBS—conducted on the Illumina Genome Analyzer machine housed at the Molecular and Cellular Imaging Center, investigators identified 27 differentially expressed candidate genes for FS8.1. Surprisingly, nearly all the genes that are differentially expressed are found at the fs8.1 locus. Moreover, with the exception of two genes, all of the fs8.1-mapped genes show higher expression in long fruit. Genetic analyses suggested that FS8.1 might be lower expressed in elongated versus round fruit. Therefore, the two genes that exhibit lower expression in elongated fruit are the best candidates to underlie the fruit shape locus. Very few genes were differentially expressed that mapped elsewhere in the tomato genome. Thus, the researchers were not able to identify known plant developmental pathways that might mediate shape changes controlled by the FS8.1 gene.

Scientists plan to verify differential expression of these two candidate genes in other plant materials. A manuscript describing these findings is in preparation. This research was conducted by Gustavo Rodriguez and Josh Clevenger of the Van der Knaap Laboratory and Asela Wijeratne, Saranga Wijeratne, and Tea Meulia of the Molecular and Cellular Imaging Center.

Esther van der Knaap

Acoustic Monitoring of Agroecosystem Rhythm, Function, and Change

Casey W. Hoy, Entomology

Conservation of biodiversity is one of the greater global environmental challenges of our time. Biodiversity—the wide variety of kinds of living things—ensures that ecosystems function in ways that provide energy, renewable materials, clean water, air, and food. But biodiversity is a very difficult thing to measure because every living thing in a particular area must be catalogued. This is quite a job when researchers estimate that there are more than 10 million different species on earth! This project was based on the idea that when there is high biodiversity, one should be able to hear it because many different kinds of animals make sounds. The purpose of this project was twofold: to develop the technology to record sounds in agricultural ecosystems over time, and to test how researchers can use those recordings to measure biodiversity efficiently and continuously.

Most previous examples of this kind of work were from oceans, where the assumption was that human sounds represent harm to the ecosystem. But agriculture is a part of the ecosystem, so in a healthy agricultural ecosystem, sounds from farming should coexist with sounds from and the natural world. If only tractors and machines are heard, then the farming system is probably too intensive to maintain biodiversity. But if one can hear the machines and also birds, insects, amphibians, and other animals all together, then the farming system is more likely to be in harmony with nature. Therefore, investigators listened for both farming and natural sounds in their recordings, but the trick remained the same: how to get from a recording to a measure of biodiversity on the farm.

Researchers acquired five automated sound recording computers from collaborators at Michigan State University. Investigators reprogrammed the computers, figured out the technical challenges, and set them up on research farms to begin recording and developing a means to analyze the recordings for biodiversity. New commercially available recording equipment—the Song Meter from Wildlife Acoustics—has since been purchased and put into use. Although these units cannot be operated through wireless communication as was the case with the initial fabricated equipment, they have now been tested and appear to be more reliable. They can also store more data, can run on less battery power, and can record for longer periods without maintenance.

Investigators have been able to produce a large collection of recorded sounds from the initial test sites. Researchers used these recordings to develop and test software to analyze the audio recordings made. The programs generate the Acoustic Habitat Quality Index, a measure of human impact on the ecosystem based on the relative amounts of natural and man-made sound in the recording, and the Acoustic Entropy and Dissimilarity Indices. The Acoustic Entropy Index describes the biodiversity at a particular location based on the number of different natural sounds in the recording. The Dissimilarity Index estimates the difference between two recordings, allowing us to compare two or more ecosystems or habitats.

Scientists ran an experiment that compared more traditional measures of biodiversity, such as one-time samples of insects from traps or bird sightings within a specified time period, and the sound-based measures. They also put the microphones in areas that, based on previous research, were expected to have relatively high and relatively low levels of agroecosystem health, of which biodiversity is a key component. The study found that the diversity of natural sounds in the recordings matched the expected amount of biodiversity based on previous work, confirming that the sound recordings can detect differences in biodiversity from place to place.

The methods being developed are new, and scientists will continue to develop them as research continues. So far, the techniques have been included in two grant proposals and will be part of at least two more. Furthermore, the recording equipment will be part of a long-term monitoring project at OARDC's Mellinger Farm. This will be the first study of its kind for monitoring changes in biodiversity as a result of changing farming practices on the farm. As researchers develop the technology further, they expect to be able to monitor biodiversity in agriculture more widely and consistently. They also expect to gain a much better understanding of how biodiversity can be increased through farming practices.

Casey W. Hoy

Development of Genetic Variation for Anthocyanin and Carotenoid Pigment Content in Native American Maize

Richard C. Pratt, Horticulture and Crop Science

Foods that are very colorful contain plant pigments that contribute to human nutrition and health. Carotenoids, the pigments responsible for the yellow and orange colors of some fruits and vegetables, have important cellular functions as precursors to vitamin A, antioxidants, immune system enhancers, and protection against age-related blindness. Anthocyanins are red, purple, and blue pigments that function as antioxidants and inhibitors of cancer cell growth.

Although modern U.S. maize varieties show little variation for kernel color, traditional farmers in the desert areas of the southwest still utilize numerous varieties of maize that display considerable variation for kernel color. However, the pigment content of these varieties has not been studied. Native Seeds/SEARCH, an organization based in Tucson, Arizona, is dedicated to saving traditional crops with potentially important traits. The objective of this research was to examine the kernel pigment content of representative southwestern maize varieties conserved by Native Seeds/SEARCH. Kernel samples were obtained from 48 entries planted in replicated nurseries at Patagonia, Arizona, and Wooster, Ohio.

Investigators examined multiple plant, ear, and kernel traits, including their total carotenoid and anthocyanin pigment content. In their respective order, the most abundant kernel types were flinty (hard), floury (soft), non-pigmented (white), and yellow. Samples with high carotenoid pigment content were not in evidence whereas some varieties contained mixtures of purple and blue anthocyanin pigments—some with high concentrations. The selected high-anthocyanin lines were crossed with a newly developed population called Ohio Blue. Because carotenoid levels were low, researchers selected lines from other breeding populations on the basis of carotenoid, oil, and protein contents. These lines were crossed in many combinations to see the relationship among traits.

Total carotenoid content in hybrid seeds between two populations was more affected by the female than the male. We also observed dosage effect according to seed endosperm formation. Total anthocyanin content in hybrid seeds was mainly affected by the female parent; therefore, we concluded the mother exerts more control over this trait.

Scientists concluded that germplasm from the desert areas of the southwest generally does not possess high carotenoid pigment content, but it does display high anthocyanin content. These varieties are quite different from Corn Belt lines. Scientists need to check the adaptability in the Corn Belt area, and they need to continue the selection procedures to develop high-carotenoid and high-anthocyanin lines so as to improve the nutritional value of maize. It is clear that higher pigment lines should be used as a female when investigators make a cross to introduce the high-anthocyanin content from germplasm into Corn Belt lines.

Richard C. Pratt

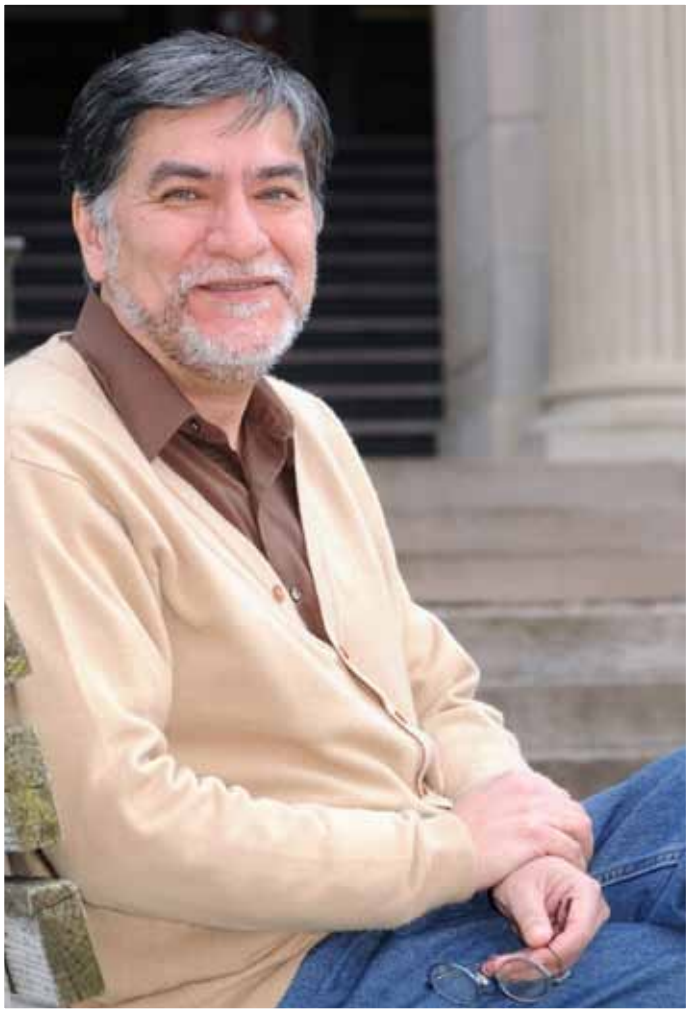
Food Insecurity and Obesity: A Paradox Emerges

Hugo Melgar-Quinonez, Human Nutrition
Joyce R. McDowell, Ohio State University Extension

It is estimated that more than 14% of U.S. households experienced food insecurity in 2008. Included among these were 5.7% of households in which food insecurity was severe due to inadequate resources of food. Based on their income-to-poverty ratio, households that are especially affected are single-mother households as well as households that are eligible as food-stamp recipients. Individuals living in households lacking in or having poor access to food often maintain diets that increase their risk of poor health, chronic disease development, and other negative outcomes such as becoming overweight and/or obese. In the U.S., the increase in the prevalence of food insecurity is parallel to the prevalence of adults who are overweight and/or obese. Almost one-third of the U.S. adult population is overweight or obese, and the numbers are growing at an alarming pace. Being overweight increases the risk of coronary heart disease, high blood pressure, diabetes, and other chronic diseases. And being obese has psychological consequences related to one's body image and emotional reactions to dieting.

Being overweight is usually thought to be associated with excessive food intake, and hunger is usually associated with an inadequate food supply. Thus, the validity of the claims of widespread food insecurity levels in the low-income population of the U.S. has been questioned because of the high prevalence of overweight people in this same population group. Although individuals with poor food security might be expected to have reduced food intake and thus have a reduced chance of being overweight, recent research in the U.S. has shown that this is not the case. Current research supports that adult women living in food-insecure households are particularly at risk of becoming overweight and obese.

Authors examining the relationship between food insecurity and obesity suggest that the relationship might be explained in part by episodic food insecurity followed by increased food intake during times of relative abundance. An abundant food supply might be available the first two or three weeks of the month followed by one or two weeks of a limited food selection due to a dwindling supply of food stamps and/or money. When money and food stamps are restored at the first of the month, food-insecure families might overeat highly palatable and rich foods, which could result in gradual weight gain over time. Although the hypothesis of a monthly cycle of food abundance and food shortage has yet to be tested, a limited number of human and animal studies provide evidence for it. These studies show that food deprivation in humans and animals and food restriction in children produce a tendency toward binge-eating behaviors when a plentiful food supply is available.



Hugo Melgar-Quinonez

This study was able to show that when resources become available at the beginning of the month, overconsumption of calories takes place among food-insecure Ohioan women. A significant decrease in caloric intake takes place by the end of the month, creating a monthly cycle of food overconsumption and food shortage. A recruitment strategy was to invite participants of Ohio State University Extension's Supplemental Nutrition Assistance Program-Education (SNAP-Ed) to be in the study. The next step in this research is to apply the preliminary findings of this study to a larger, more comprehensive research proposal that will include the development of nutrition education curricula for SNAP-Ed participants through the OSU Extension system.

Building the System to Efficiently Screen for *Hosta Virus X* Resistance

Dennis Lewandowski, Plant Pathology

Hostas are one of the most popular perennials grown in the U.S., and Ohio is a major producer of finished hostas. Recently, a high percentage of many popular hosta cultivars exhibited symptoms of virus infection. Some large growers in Ohio destroyed thousands of *Hosta virus X* (HVX)-infected hostas that were ready for sale. It also appeared that secondary spread was occurring in Ohio nurseries. Virus-infected hostas cannot be cured, so it was necessary to take a multifaceted approach to managing the virus—one that included educating growers. Although many Ohio hosta growers have been adversely impacted, this disease is not restricted to Ohio or the U.S.

The cause of this virus outbreak was a relatively recently discovered virus, HVX. At the time, the scientific community lacked the information (e.g., virus diversity, its prevalence, and host resistance) that was necessary to make science-based recommendations to growers. As we have learned more about HVX, we have been able to better educate growers. Many have bettered their sanitation practices, have proactively screened incoming shipments, and have begun to recognize suspect plants.

For this project, nursery surveys were conducted to determine which cultivars were infected. Different symptom patterns were confirmed to be associated with HVX infection. Different cultivars were screened for susceptibility or resistance to HVX. We looked at genetic diversity of HVX isolates collected from different cultivars and locations. We developed a system where we can manipulate the HVX genome to better understand the virus biology. In the greenhouse, we screened twenty hosta cultivars for susceptibility to HVX. We identified cultivars in which the virus rapidly spread into upper leaves, cultivars in which HVX spread only in subsequent growing seasons, and cultivars in which infection was localized. Importantly, HVX was not detected in several cultivars after the first summer; these cultivars may represent a source of resistance to HVX, as the four cultivars are related. These cultivars could provide breeders a source of HVX-resistance to incorporate into their breeding programs. The genetic sequence of the coat protein (CP) gene from HVX isolates collected throughout Ohio was compared to all publically available sequences to look for diversity.

Dennis Lewandowski

We found very little CP sequence difference among the isolates, which suggests that HVX is very uniform. However, an alternative interpretation is that our methods were unable to detect more diverse HVX isolates. We chose one isolate (HVX-37) for much more extensive study, and we obtained the complete genome sequence of HVX-37. Compared to an isolate collected in Korea, there was a variable region of the HVX-37 genome. However, we cannot exclude that these differences were sequencing artifacts. HVX is an RNA virus, and we constructed an artificial DNA copy of HVX-37, thereby extending its life cycle through an artificial DNA phase. From the DNA copy, researchers can reproduce infectious HVX in the laboratory. This gives investigators a powerful reverse genetics system for HVX; researchers can manipulate the genome as DNA, make RNA in the lab, inoculate hostas, and ask what effect these changes have on the biology of the virus (e.g., transmission, packaging, movement, symptoms, etc.).

Future plans include genetically modifying the HVX clone to enable HVX to express a fluorescing protein (GFP). This will enable scientists to monitor virus infection without destructively sampling the plant. This will enable us to rapidly screen hostas for resistance to HVX. Plants that do not fluoresce in response to infection by HVX-GFP will be candidates for more intensive screening for resistance using serological and nucleic acid-based methods.

Ultimately, growers will be able to realize increased profits over time by destroying fewer diseased plants. Consumers will have a greater supply of healthy plants from which to select. Hosta enthusiasts will minimize potential risks to their hosta collections from this pathogen.

What Does Agriculture Have to Do With It? Critical Thinking Skills of Agricultural Communication-Trained Versus Journalism-Trained Media

Emily Rhoades, Human and Community Resource Development

Over the last ten years, science-heavy topics have exploded as front-page news. As more science and research is reported, it has become more necessary than ever for reporters to ensure fair and accurate coverage of topics. Agriculture is a major industry in the state of Ohio, so the inaccurate handling of such issues in the media can have detrimental economic effects. The purpose of this study was to explore the relationship between journalists' critical thinking skills and their experiences in agricultural and environmental sciences. Findings of this research will not only further research in critical thinking, but will help in the development of stronger journalistic curriculum so that agricultural and mainstream journalists will be better prepared to report on topics in agriculture and environmental sciences in Ohio and across the country.

The researchers of this study hypothesized that journalists who have been exposed to complex scientific topics in their education and careers (like those in agricultural and environmental sciences) are more disposed to thinking critically. Survey methodology was implemented to a random stratified sample of approximately 500 media professionals working for general and agricultural media organizations. Populations included agricultural journalists who are members of the American Agricultural Editors Association and newspaper journalists listed in the Gebbie Press directory for the states of Ohio, New York, Georgia, Louisiana, Oregon, Colorado, Texas, and New Mexico. The sample of 75 dailies per state was stratified based on geographic location, in an attempt to have representation from across the country.

After six waves of contact, 96 media categorized as being agricultural responded and 29 media categorized as being mainstream responded. (Due to the nature of the profession, media have been noted to be a difficult population to reach.) When asked about their experiences with agriculture, the agricultural journalists indicated having had college classes focusing on the industry (56.4%, n=53) while 86.2% (n=25) of mainstream journalists reported no courses in agriculture during their college education. Further questioning on reporting showed that, of the mainstream reporters responding,

Emily Rhoades

65.6% (n=19) did not have a special agriculture section in their papers; however, 67.9% (n=19) said they had reported on agriculture at some point in their career.

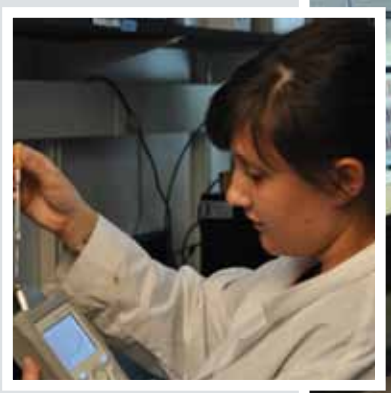
Respondents were asked about their knowledge of agriculture and their background with the industry. Agricultural journalists reported having an average to above average knowledge of the industry; however, 44.4% of mainstream journalists reported having an average level of agricultural knowledge. Further, 38.5% feel that they have an average experience with agriculture, while 57.7% feel that they have an average to above average ability to cover agriculture issues. Further analysis indicated, not surprisingly, that there is a moderate correlation between being an agricultural reporter and being confident in the ability to report agriculture.

The majority of agricultural media in this study (86.9%) indicated that they agree to strongly agree that agriculture does a good job of informing the public on the industry. The majority of mainstream reporters differed, with 50% saying they disagree with the statement. However, the majority of both groups (agriculture: 42.9%, mainstream: 60.7%) agree that the agriculture industry does an average to above average job in public relations with the media.

Findings are currently being shared with professional and academic journals in science and communication on how to work with journalists to ensure that accurate sources are used and that accurate information is synthesized effectively in today's media.

Student Projects

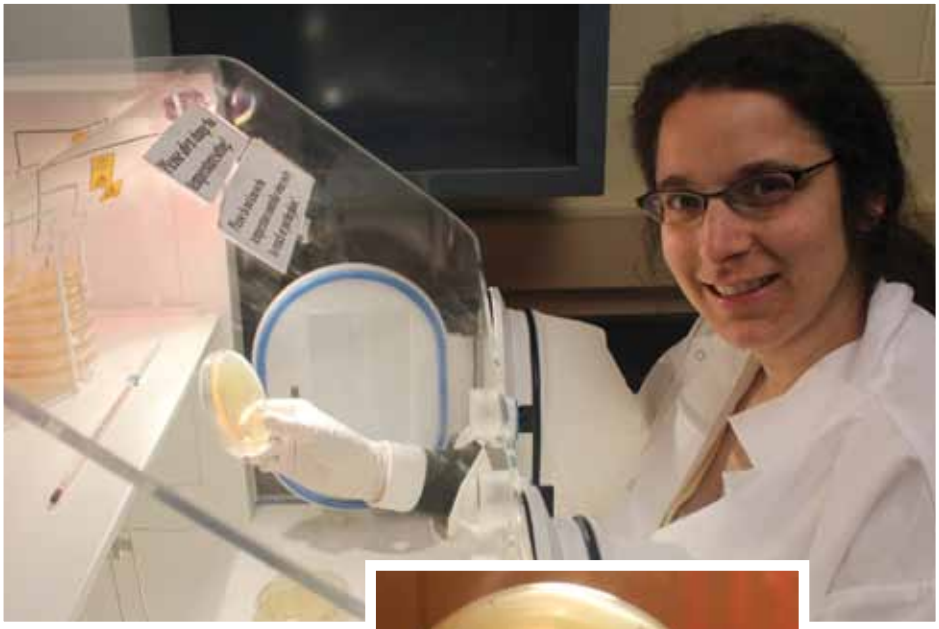
The Director's Undergraduate Research Competitive Grant Program, funded to a maximum of $3,300 per award, provides undergraduate students with a professional grant-writing, research, and reporting experience. Projects are designed, submitted for review, and carried out with a faculty mentor. Once a project is completed, students take an independent studies class to write their research report in the form of a scientific journal article, using their faculty advisor as an editor. Some of these reports get published, and many students present their research at professional meetings and competitions. The Graduate Research Competitive Grants Program offers two categories of funding. Doctoral students may receive up to $5,000 per award and master's students up to $3,000 per award. Graduate students who receive awards are asked to serve on a panel to review applications in the following year's competition. This experience provides students with the opportunity to develop their skills in grant-writing and reviewing—skills that are essential to their careers.



Small Molecule Inhibitors as Anti-*Campylobacter jejuni* Drug Candidates

Mary Drozd, Food Animal Health Research Program
Gireesh Rajashekara, Food Animal Health Research Program
SEEDS Doctoral Competition

Mary Drozd



A bacteria known as *Campylobacter jejuni* causes a very common foodborne gastrointestinal disease in humans. The most common way that people become infected is from eating contaminated chicken meat. This bacteria colonizes the intestinal tract of food animals and often contaminates the chicken carcass via fecal exposure during slaughter. *Campylobacter* contamination of chicken meat is very common in the U.S.; it has been estimated that 80% of commercial poultry meat is contaminated. While *Campylobacter* causes a globally prevalent illness, Ohio produces more than 300 million pounds of chicken meat annually. Therefore, *Campylobacter*-related diseases are important to the health of the Ohio poultry industry as well as to its customers.

This study targets the ability of *Campylobacter* to establish persistent colonization in chickens. Previously, it was found that the Twin-Arginine Translocation (TAT) transports more than 40 molecules important for the survival of *Campylobacter* in chickens. Scientists have designed a high-throughput assay that will allow screening for small-molecule chemicals that inhibit the TAT system. A small-molecule chemical has several advantages as a potential therapeutic, including its abilities to passively enter bacterial cells and interfere with a single, specific biological reaction. In order to find a small-molecule chemical that interferes with the TAT system, investigators designed an assay that allows for measurement of the effectiveness of TAT transport. When the cells are treated in this assay with small-molecule candidates, scientists will be able to detect those that affect the ability of *Campylobacter* to use the TAT system. Scientists also developed secondary assays, allowing for the elimination of any false positive results from the initial screen.

The application to collaborate with the Harvard NSRB facility was recently accepted. Researchers plan to visit the NSRB facility to conduct a screening of their small-molecule library this summer. Once researchers find a primary group of small-molecule candidates, they will select those chemicals using the small-molecule secondary screens. There will also be cross-college research with The Ohio State University Department of Chemistry to collaborate with current investigators to produce small molecules for further testing.

Assessing the Efficacy of Fresh Produce Sanitizers Using De-Correlation Technology

Melanie Lewis Ivey, Plant Pathology
Sally A. Miller, Plant Pathology
SEEDS Doctoral Competition

The contamination of fresh produce by zoonotic pathogens is not a new problem; however, recent advances in pathogen detection, identification, surveillance and dissemination of information, as well as the occurrence of several large-scale outbreaks has led to increased public awareness about foodborne illnesses associated with fresh produce. The latest statistics from the Centers for Disease Control and Prevention (CDC) estimate that 1 in 6 Americans becomes ill and 3,000 Americans die each year from foodborne pathogens. CDC also states that 90 percent of estimated illnesses, hospitalizations, and deaths are due to just 7 pathogens: *Salmonella*, *Norovirus*, *Campylobacter*, *Toxoplasma*, *Escherichia coli* O157, *Listeria*, and *Clostridium perfringens*. In 2004, the Alliance for Food and Farming reported that 12% of all foodborne illnesses are associated with the consumption of fresh fruits and vegetables. While there are no standards for acceptable levels of *E. coli* O157:H7 and *Salmonella* species on fresh produce in the U.S., current USDA guidelines for acceptable levels of generic *E. coli* O157:H7 and *Salmonella* species on ready-to-eat foods state that these organisms should not be present in 25g of food.

While very little is known about the biology of these pathogens on fresh produce, it is known that eliminating these pathogens once they are on the produce is extremely difficult and usually unsuccessful. Many different post-harvest disinfectants and methods of application have been evaluated over the last 10 years, with little to no success. Furthermore, it has been shown that these bacterial pathogens can adhere to and move on the plant or fruit surface using very different mechanisms, suggesting that the effectiveness of one treatment or method of application may vary depending on the pathogen type. Nonetheless, finding one method and/or disinfectant to kill multiple types of pathogens on fresh produce would be an economically feasible strategy for vegetable producers.

In this study, de-correlation technology (Smart Fog) was used to apply two commercially available disinfectants to contaminated lettuce and tomatoes and non-contaminated grapes. The Smart Fog Tunnel Treatment System is a humidification system that generates microscopic droplets of sanitizer with equal spacing; the droplets oxidize in the air rather than attract to each other. The system is designed to attach to a grower's existing conveyor belt system. As the produce passes through the tunnel, it is covered with a dense fog of disinfectant. Hydrogen dioxide (StorOx, BioSafe Systems) and didecyl dimethyl ammonium chloride (KleenGrowTM, PACE 49 Inc.) applied at multiple application rates, exposure times, and densities were tested for their ability to kill *E. coli* O157:H7 on lettuce, *Salmonella enterica serovar Typhimurium* on tomatoes, and coliform bacteria on grapes. Neither disinfectant was effective at killing *E. coli* O157:H7 on whole-head lettuce or freshly chopped lettuce, and *E. coli* O157:H7 populations were higher on chopped lettuce samples than whole-head samples even after sanitation. Coliform bacteria on nondisinfected grapes were not detected; however, they were detected on grapes disinfected with hydrogen dioxide (moderate and high rates). When applied at high rates, both disinfectants were effective at eliminating *S. enterica serovar Typhimurium* from the surface on fresh tomatoes.

Continued research on the effectiveness of hydrogen dioxide and didecyl dimethyl ammonium chloride as well as other potential disinfectants in killing human bacterial pathogens on fresh produce is needed. While didecyl dimethyl ammonium chloride was effective at killing *S. enterica serovar Typhimurium* it was not effective at killing *E. coli* O157:H7 on lettuce. Because vegetable producers process multiple types of vegetables in a growing season, finding disinfectants that target multiple pathogens—including post-harvest plant pathogens—would be of economic benefit to the producer and processor and could have great potential economic impact to the industry as a whole.

Melanie Lewis Ivey

The Roles of Three Unique Tandem Zinc Finger (TZF) Genes in Seed Development and Germination in *Arabidopsis thaliana*

Srimathi Bogamuwa, Horticulture and Crop Science
Jyan-Chyun Jang, Horticulture and Crop Science
SEEDS Masters Competition

Tandem Zinc Finger (TZF) genes are important for gene regulation and hormone response in humans. Researchers have identified three TZF genes (TZF4, TZF5, and TZF6) highly expressed in *Arabidopsis* seeds. Based on available literature and preliminary results, scientists hypothesize that TZF4-6 are involved in seed development, dormancy, and germination. Seeds are the most important propagates and storage units of crops and ornamental plants. Seed dormancy is the inert stage of plant life cycle. It prevents or delays germination. However, dormancy increases shelf life of harvested seeds. In plants, dormancy helps seed development by preventing premature germination. Hence, dormancy is a very important property that affects seed quality. Understanding the functions of TZF4-6 genes during seed development and germination is expected to improve seed quality.

Researchers have found that both TZF4 and TZF5 are expressed in dry, dormant seeds, and their expression levels decreased during hydration. During seed hydration, in general, plant hormone abscisic acid (ABA) levels go down and gibberellic acid (GA) levels go up. Therefore, ABA and GA regulate seed dormancy and germination antagonistically. Scientists speculated that TZF4 and TZF5 are induced by ABA and repressed by GA. TZF6 only expresses in young and mature seeds, raising a possibility that this gene is involved in seed development.

To test these hypotheses, investigators created promoter-reporter transgenic plants of these genes. Promoter is the part of the gene that controls the gene expression. The promoter of each gene was fused with GUS reporter gene. When the fusion gene is expressed, GUS reporter gene produces a blue color. Transgenic plants were used to determine where, when, and under what environmental and hormonal conditions these genes will be expressed. To investigate how these genes affect seed germination and plant growth and development, researchers identified loss-of-function and created gain-of-function plants of these genes. Seed germination assays were performed under different environmental conditions. For instance, I used high concentrations of sodium chloride to mimic the salt stress.

When these genes were knocked out, all mutants showed early germination. By contrast, gain-of-function seeds were hypersensitive to high concentrations of sugar and salt. Based on this study's results, scientists suggest that these genes may act as negative regulators of seed germination. To support this notion, investigators found that TZF4-6 are expressed in radical region of the embryo in the presence of ABA. Seed germination is defined by the emergence of the radical through seed coat. This expression pattern is similar to other known negative regulators of seed germination. Further investigation of the significance within this unique expression pattern is planned.

Srimathi Bogamuwa

Sedimentation and Erosion Patterns in a Fifteen-Year-Old Created Wetlands

Sara McCarthy, School of Environment and Natural Resources
William J. Mitsch, School of Environment and Natural Resources
SEEDS Undergraduate Competition

Sedimentation is an important process in wetlands. It improves water quality, increases water clarity, submerses plant accessibility to sunlight, and retains nutrients that otherwise cause eutrophication downstream. By studying sedimentation in wetlands, increased sediment retention can be achieved through enhanced created and mitigated wetland design.

The Gulf of Mexico contains anoxic dead zones that are arguably caused by high nitrogen outputs from farms along the Mississippi River and adjoining watersheds. This has drastic economic consequences for the fishing industry of the Gulf region. Increased knowledge of sedimentation in wetlands can aid in the sequestration of nitrates from farms; this will help improve the aquatic ecosystem of the Mississippi watershed, allowing for increased species diversity. As contaminants are removed from rivers, nearby inhabitants will have improved health benefits. Wetlands are known to remove heavy metals such as zinc and cadmium through sedimentation and chemical processes that can be harmful to humans upon ingestion. The ability to reduce sediment as much as possible from rivers can also reduce odor and increase aesthetic viability.

As researchers uncover more about the impacts of spatial variables on sedimentation processes, wetlands can be designed in ways that sequester an increased amount of nutrients. This will lead to cleaner river water, which regenerates riverine biotic populations (fish, macrophytes, macroinvertibrates, etc.), restores ecological function of the watershed, and has health and aesthetic benefits for nearby human inhabitants. This study will have a definite impact on the future design of wetlands and their ability to sequester nutrients and sediment.

The main problem addressed was to quantify net sedimentation in two 15-year-old, man-made riverine wetlands at the Olentangy River Wetland Research Park. This was done by laying a feldspar horizon layer, allowing sediment to accumulate over a one-year period, and then measuring the depth of sediment by means of a cryogenic soil corer and micrometer. In addition to obtaining net sedimentation data, it was the goal of this research to create an accurate sediment budget for the two wetlands.

Sara McCarthy

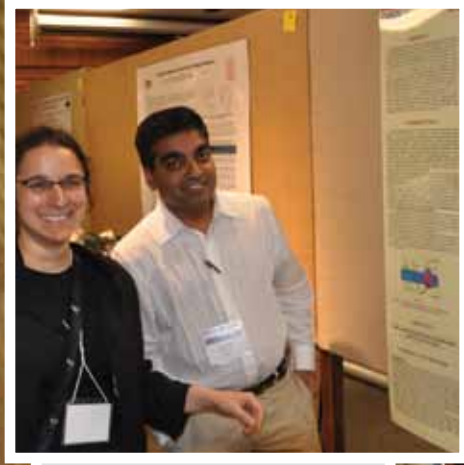
Sediment flux data was calculated from inflow and outflow data with respective total suspended solids data. Erosion was measured every season at 12 sites by estimating the change in elevation of the sediment surface in comparison with the stable boardwalks. Biological impacts on sedimentation were quantified using wildlife exclosures on 8 of the 32 sedimentation sites. Sites with exclosures were compared to those without exclosures to quantify the effects of fish, amphibians, birds, and small mammals.

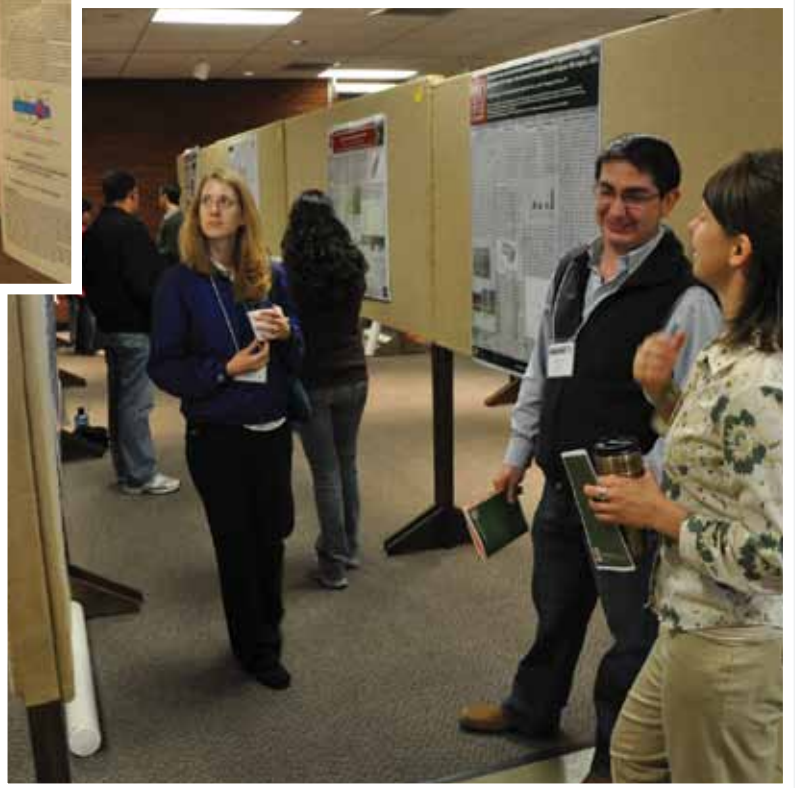
Based on preliminary findings, we found significant seasonal variation in erosion rates in the two wetlands. Erosion rates from May–July were statistically different from those measured from July–October. In late summer, erosion in the naturally colonizing wetland was 0.01–0.02cm/day while sedimentation of 0.07–0.02cm/day occurred in late spring. Similarly, the planted wetland experienced net erosion of 0.005–0.02cm/day in late summer, with net sedimentation of 0.05–0.02cm/day in the late spring. Erosion impacted gross sedimentation rates for 83% of the erosion sites studied.

Additional findings from this study will be published in an Ohio State University master's thesis and an environmental journal in 2011. The explanation of spatial variants (distance from inflow/outflow, vegetated vs. open water, etc.) and their effects on sedimentation rates will aid future wetland creators in designing wetlands; thus, more sediment can be sequestered. The work of determining sedimentation changes with wetland age will continue at the Olentangy River Wetland Research Park as this experiment is repeated and methods are improved upon in time.

Publications, Presentations, and Graduate Students

Sharing knowledge through publications and professional meetings is an important part of research, as is training graduate students for careers in research. Using data from SEEDS projects, OARDC scientists have reported the publication of 578 peer-reviewed articles, bulletins, abstracts, and popular press articles. More than 1,000 presentations have been made in locations throughout the world. Eighty-five graduate students have been supported on SEEDS projects, thus being provided with the skills needed to move forward with scientific research in the future.





Presentations

Allendorf, M., and Rodriguez-Saona, L.E. 070-19. Application of Handheld Portable Infrared and Raman Sensors for Characterizing and Monitoring Quality of Edible Oils. Institute of Food Technologists Annual Meeting. Chicago, IL. July 2010.

Allendorf, M., and Rodriguez-Saona, L.E. 1690-3P. Application of a Handheld Portable Infrared Sensor for Monitoring Oil Quality. Pittcon. Orlando, FL. March 2, 2010.

An, R., and Grewal, P.S. Cooperative Endurance: A Remarkable Strategy Adopted by Symbiotic Bacteria to Persist in Their Nematode Vector. OARDC Annual Research Conference. Columbus, OH. April 28, 2011.

An, R., and Grewal, P.S. Differential Gene Expression of Bacteria Reveals Strategic Symbiosis with Nematode. Wooster Area Molecular Biology Association (WAMBA). Wooster, OH. November 14, 2008.

An, R., and Grewal, P.S. Molecular Mechanism of Symbiosis Between Entomopathogenic Nematodes and Bacteria. The Joint Meeting of the Society of Nematologists and Soil Ecology Society. Burlington, VT. July 12–15, 2009.

Bae, D., Shin, J., Reddish, J.M., Latshaw, J.D., Wick, M.P., and Lee, K. The Study of Gene Delivery System in Avian Species Using Human Adenoviral Vector. Annual Animal Science Meeting. Indianopolis, IN. July 2008.

Birkel, E., and Rodriguez-Saona, L.E. 070-02. Detection and Quantitation of Trans Fat in Fats and Oils Using a Portable Handheld Infrared Spectrometer. Institute of Food Technologists Annual Meeting. Chicago, IL. July 2010.

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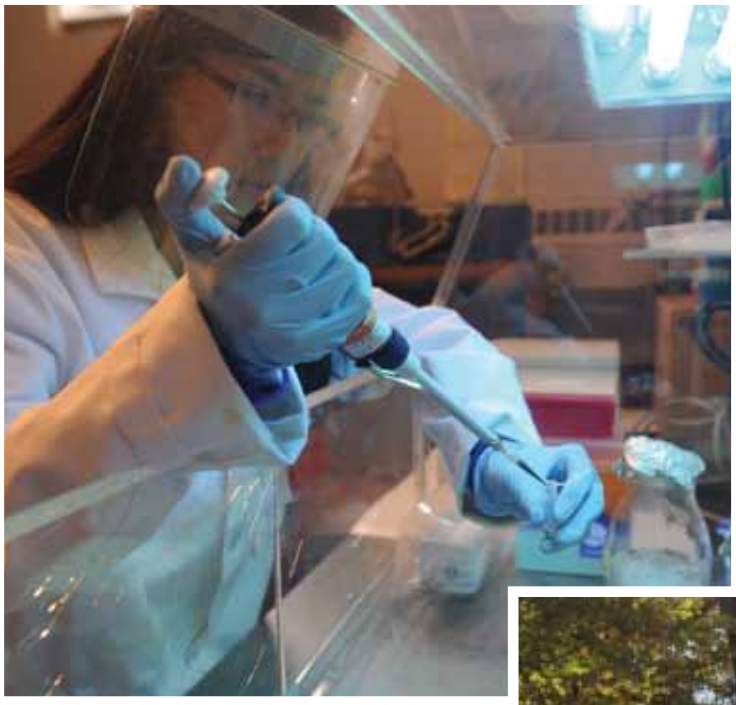

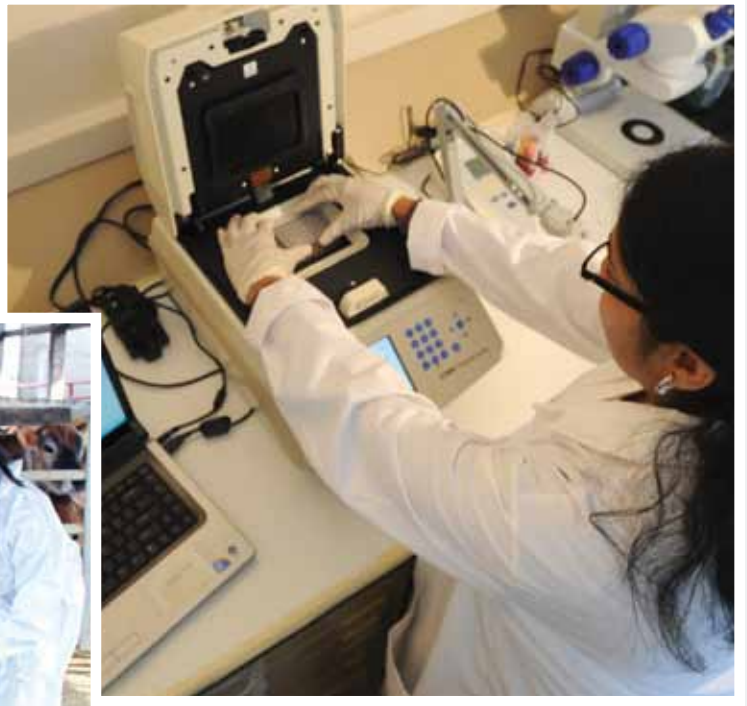

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